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THE EFFECT OF PHYSICAL TRAINING ON OXYGEN DEBT AND GLYCOGEN RESERVES IN TROUT[1]

P. W. HOCHACHKA[2]

Abstract

Rainbow trout, *Salmo gairdneri*, conditioned to fast-flowing water, differ from untrained fish in several ways. The trained fish are able to acquire an oxygen debt three times as large as the untrained ones. Almost twice as much of the available glycogen in muscle can be used up during short bursts of swimming. The trained fish have higher levels of blood haemoglobin and relatively larger hearts. Following fatigue, the recovery of glycogen in muscle and liver is more rapid. The data suggest a modus operandi for short but severe bursts of swimming. The two critical factors are: (*a*) initial energy supplies and (*b*) tissue buffers. The former preclude the exhaustion of the muscle; the buffers allow a large utilization of the fuel, i.e., a large excess of lactic acid and, consequently, a large oxygen debt. Since even in trained trout only 70% of the muscle glycogen is used, fixed acid probably limits the stamina in both groups of fish.

Introduction

Washbourn (20) showed that the resting respiration of fish taken from swift currents is higher than that of fish from slow-moving waters, but the reasons for this have remained obscure. In a different approach, Brett *et al.* (6) found that salmon are able to maintain a faster cruising speed after they have been physically conditioned to a strong water current. With the exception of these two studies, there have been no serious attempts to work out what changes, if any, are incurred by training fish physically, although the influence of training on mammalian physiology is known in some detail (9, 12). By comparing two groups of trout, one conditioned to swift-flowing water, the other to a very slow current, this study attempts to evaluate the training effect in fish.

Experimental Fish

A group of rainbow trout fingerlings, *Salmo gairdneri*, chosen at random from a stock held at the Calgary Trout Hatchery, was placed in a rectangular tank, 27 ft long and 5 ft wide, with an average depth of 2 ft and an average surface velocity of about 0.04 ft per second. These fish were the *untrained* lot. Another group was held in a circular tank of 12-ft diameter, ranging in depth

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from 0.7 ft near the edge to 2 ft in the middle, and with a surface velocity of about 1 ft per second. These were the *trained* trout.

Both groups were fed Clark's dry rations (14) at equal rates. The same water supply, almost saturated with oxygen and at 4° C, was delivered to both tanks. The fish in the circular tanks orientated into the current and tended to maintain their positions constant despite the rapid water flow. In the rectangular tank, the trout did not take up any characteristic positions.

On a growth basis, the two lots of trout were similar (Mr. A. C. Sinclair, personal communication):

	Trained	Untrained
Initial weights, g	1.9	3.4
Final weights, g	21.8	25.2
Mortality, %	5.4	4.1
Food conversion factor (lb food/lb fish)	2.0	1.9

With water velocity being, thus, the only known variable, the two lots of fish were kept under these conditions for 6 months before they were compared.

Materials and Methods

Oxygen debt was measured by determining the oxygen consumption (*a*) during routine metabolism (7) and (*b*) immediately after the fish were exercised to fatigue and continuing up to the time at which routine metabolism resumed. The trout were fatigued by chasing them in troughs 27 ft long, 1 ft wide, and about 0.5 ft in depth. Following exercise, each fish was transferred to a respirometer.

The respirometers were modified polyethylene utility boxes with airtight lids. An inlet tube extended to the end of each box to ensure an even circulation of water; an outlet tube extended to a 25-ml collecting flask, which overflowed into a graduated cylinder. Since the boxes were submerged when in use, extra openings were made on the tops to allow for the removal of air bubbles. Water flow through each respirometer could be regulated easily by changing the level of the collecting flask. Oxygen was determined in both inflowing and outflowing water by a Winkler microtitration (Dr. C. P. Hickman, personal communication).

Glycogen levels in liver, heart, and epaxial muscle were determined under three standard conditions: (*a*) with fish in the unexercised state, (*b*) immediately after fish were exercised to fatigue, (*c*) at various periods after fish were exercised.

The trout were killed by decerebration and blood for haemoglobin assay was drawn from the head wound. Haemoglobin was determined by the Haden-Hausser acid–hematin method. Whole hearts and small samples of liver and muscle were excised, then placed directly into weighed test tubes containing 30% KOH. Sampling was completed within 60–90 seconds. Glycogen was assayed by the phenol – sulphuric acid method (15).

Results

Behavior of Trout in Respirometers and during Exercise

When unexercised trout were placed in the respirometers, they followed a typical exploratory behavior. After some minutes, they would assume a resting

position, but this type of activity was repeated periodically. Hence, true resting O_2 intake could not be determined. The rates given for unexercised trout probably represent Fry's (7) "routine" metabolism.

All trout could be fatigued within 5 minutes of the onset of exercise. During the first 1–3 minutes, a typical escape response occurred. Later, however, attempts at hiding and resting began to replace the normal escape efforts. By 5 minutes of exercise, the fish were exhausted and showed no response to further stimulus. At this time, they were either sampled or transferred to the respirometers.

Differences in stamina between the trained and untrained trout were noted early in the study. A comparison of 15 trout from each lot yielded the following averages: 4.1 minutes to fatigue for the trained lot and 2.6 minutes for the untrained fish.

Oxygen Consumption in Unexercised and Exercised Trout

The oxygen intake of all unexercised trout was essentially the same (Table I). The rise in uptake that occurred during the first 4 hours can be ascribed to activity in the hand nets while the trout were being transferred to the respirometers as well as to the activity within same.

TABLE I

Oxygen intake of trained and untrained rainbow trout before and after exercise, expressed in mg O_2/l./hr/g

Group	Hours after exercise	Average O_2 intake	
		After exercise	Unexercised
Trained	1	0.110±0.014* (6)	0.094±0.005 (6)
	2	0.182±0.016 (10)	
	3	0.142±0.007 (4)	0.112±0.012 (6)
	4	0.144±0.011 (6)	
	6	0.100±0.011 (8)	0.096±0.010 (6)
Untrained	1	0.103±0.006 (3)	0.097±0.013 (6)
	2	0.108±0.007 (6)	0.097±0.011 (6)
	3	0.130±0.008 (6)	0.117±0.004 (6)
	4	0.137±0.022 (6)	0.122±0.006 (6)
	6	0.111±0.006 (6)	0.097±0.013 (6)

Numbers in parentheses refer to numbers of fish tested.
*Plus or minus the standard error of the mean.

The extent of oxygen debt is determined by the difference between oxygen consumption after fatigue and that which would have been consumed in the unexercised state (9). In Fig. 1, the areas between the respiration curves of the exercised and unexercised trout are quantitative expressions of oxygen

debt. By determining these areas for both groups, it can be seen that trained rainbow trout acquired an oxygen debt up to three times the size of that accumulated by the untrained ones.

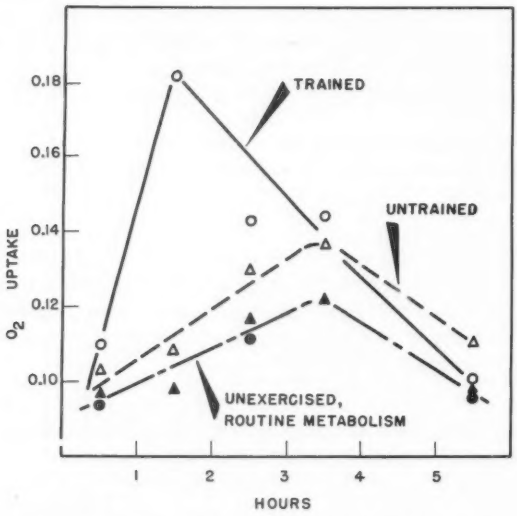

FIG. 1. Oxygen consumption by trained and untrained rainbow trout in mg/l./hr/g. The lower curve represents the average respiration of unexercised trout from both groups; the upper two curves, the payment of an oxygen debt incurred by severe exercise. The number of measurements at each point is the same as in Table I.

Muscle Glycogen

Table II and Fig. 2 summarize the changes in muscle glycogen following fatigue. Only during routine metabolism did trained and untrained trout show similar concentrations of glycogen in their epaxial mass. The percentage decrease at fatigue in the trained lot was about 65–70%, but, in the untrained fish, it was only about 40%. That is, the trained fish were able to utilize more of the available glycogen in muscle and this may, in part at least, account for their superior stamina. A consequence of this would be a larger excess of lactic acid, and hence, a greater oxygen debt.

Recovery varied with training as well (Fig. 2). Redeposition of glycogen in muscle of trained fish began shortly after the end of exercise; in the untrained lot, very little recovery occurred even after 10 hours of rest.

Blood glucose and lactic acid may contribute to restoration of glycogen in muscle. Meyerhof and his co-workers showed that in frog muscle four-fifths of the lactic acid was converted to carbohydrate, the remainder being oxidized. Calculations based on the assumption that all the glycogen used during short bursts of swimming is hydrolyzed to lactic acid yield about 1/6 as an estimate of the amount of lactic acid that can be oxidized in trout. The rest, presumably, is converted to glycogen or fatty acids (4).

TABLE II

Tissue glycogen of trained and untrained rainbow trout, before and after exercise, expressed in mg/100 g tissue

Material	Group	Unexercised	At fatigue	Time after exercise: 15 min	1 hr	4 hr	6 hr	10 hr
Muscle glycogen	Trained	174 ± 12* (9)	61 ± 14 (5)	72 ± 5 (3)		69 ± 29 (4)	84 ± 15 (7)	111 ± 5 (5)
	Untrained	178 ± 20 (5)	107 ± 16 (5)		49 ± 9 (3)	44 ± 17 (3)	62 ± 4 (6)	52 ± 4 (5)
	Wild *S. clarki*	226 ± 24 (29)						
Liver glycogen	Trained	4132 ± 325 (9)	4120 ± 652 (5)			2572 ± 319 (4)	2435 ± 278 (6)	3146 ± 473 (5)
	Untrained	4994 ± 712 (5)	5110 ± 738 (5)		2423 ± 258 (3)	2126 ± 737 (3)	2455 ± 269 (6)	1664 ± 269 (3)
	Wild *S. clarki*	4490 ± 280 (39)						
Cardiac glycogen	Trained	541 ± 47 (9)	599 ± 66 (5)	783 ± 113 (5)	675 ± 40 (3)	642 ± 119 (4)	643 ± 75 (4)	619 ± 61 (4)
	Untrained	782 ± 78 (5)	611 ± 58 (5)		844 ± 60 (4)	327 ± 23 (3)	644 ± 92 (6)	613 ± 35 (3)
	Wild *S. clarki*	911 ± 77 (41)						

Numbers in parentheses refer to number of fish tested.
*Plus or minus the standard error.

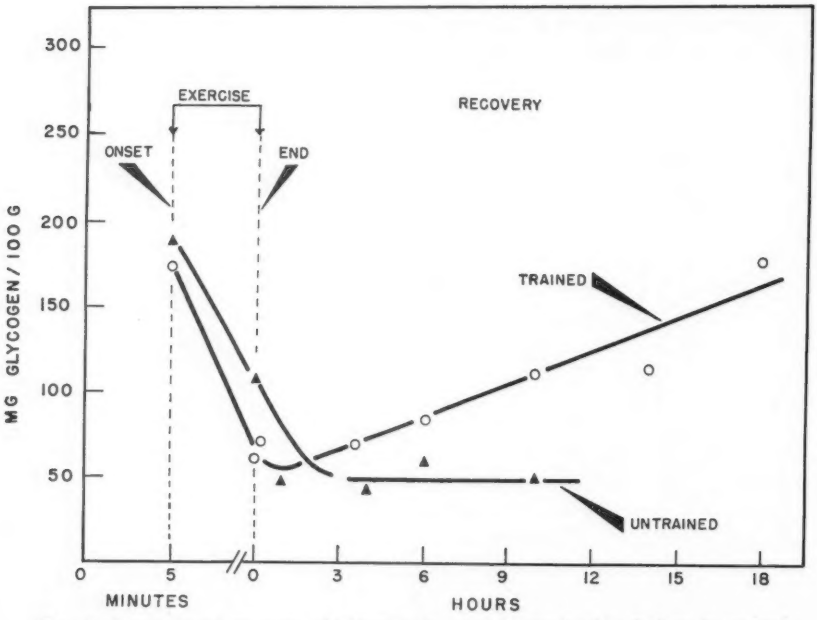

FIG. 2. The fall and recovery of glycogen in epaxial muscle after fatigue in trained and untrained rainbow trout. Each point is an average of three to nine analyses.

Liver Glycogen

Changes in liver glycogen following exercise are recorded in Table II. This material did not fluctuate as violently as muscle glycogen. Differences between trained and untrained trout were not marked. Recovery in the untrained lot probably was slower: at 10 hours after exercise, the trained trout had concentrations almost twofold higher than the untrained fish. These data agree closely with those of Miller *et al.* (14) and Black *et al.* (3).

Cardiac Glycogen

Untrained trout had higher concentrations of heart glycogen than did the trained ones (Table II). Following fatigue, there seemed to be a gradual rise in these deposits, but this was not entirely consistent and possibly was due to some secondary factors such as circulating adrenaline (5). It is not surprising that the changes in cardiac glycogen after exercise were slight, since one would not expect so favored a system as the cardiovascular one to be strongly affected by only a short burst of activity.

Tissue Glycogen in Wild Cutthroat Trout, Salmo clarki

The levels of glycogen in the heart, liver, and muscle of wild cutthroat trout are recorded in Table II for the sake of comparison. These levels were as high as, or higher than, those observed in the hatchery-reared rainbow trout. A field study (11) recently has summarized the consequences after the stream planting of hatchery fish.

Blood Haemoglobin

Trained rainbow trout had significantly more haemoglobin in their blood than untrained ones (Table III). And 1- to 3-year-old wild trout had higher levels than 3- to 5-year-old ones. Some unpublished data of the writer indicate a similar age-trend in hatchery fish.

TABLE III

Levels of blood haemoglobin in trained and untrained *S. gairdneri*, expressed in g/100 ml of blood. Wild *S. clarki* were sampled in midsummer

Group	No. tested	Age, years	Haemoglobin
Trained	10	1	11.4±0.25*
Untrained	10	1	9.1±0.20
Wild stock			
S. clarki	5	1–3	12.0±0.33
S. clarki	5	3–5	11.3±0.18
S. gairdneri	15	1–3	11.9±0.20

*Plus or minus the standard error.

Heart Size

Table IV summarizes the data on relative heart sizes in the trained and untrained rainbow trout as well as in wild *S. clarki*. The wet weight of the heart was multiplied by 100 and expressed relative to the wet weight of the body. Treated in this way, the data show that the trained trout had relatively larger hearts than the untrained ones. Wild stock males had somewhat larger hearts relative to body weight than females. Contrary to the case with the young hatchery fish, the sample of wild trout included fish with mature gonads, so that the sex difference can probably be explained on the basis of ovary and testis weights. More notably, all the wild stock had heart-to-body ratios that were significantly higher than in either of the hatchery lots. If Starling's law of the heart, that mechanical energy set free on passage from the resting to the contracted state depends upon the length of the muscle fibers, is still accepted (18), the advantage again seems to be on the side of the trained and wild trout.

TABLE IV

Heart size in trained and untrained *S. gairdneri*. Wild *S. clarki* were collected in midsummer. Wet weight of the heart was multiplied by 100 and expressed relative to the wet weight of the body

Group	No. tested	100×heart weight/body weight
Trained	50	0.133±0.003*
Untrained	25	0.119±0.003
Wild stock		
S. clarki, males	25	0.180±0.008
S. clarki, females	21	0.151±0.005

*Plus or minus the standard error.

Discussion

Although lactic acid was once thought to indicate hypoxia and the formation of an oxygen debt, the original suggestion being that of a close relation between lactate production and oxygen deficiency (10), this is no longer acceptable in its simplest form, for lactate concentration is controlled by two factors, pyruvate concentration and the state of oxidation of diphosphopyridine nucleotide (DPN):

$$(\text{lactate}) = (\text{pyruvate}) \times K(\text{DPNH})/(\text{DPN}).$$

Since the DPNH/DPN ratio is oxygen-dependent, it is clear that pyruvate changes can affect lactate levels as much as oxygen lack (12). Thus only when a lactate change exceeds that which is appropriate to the pyruvate alterations need oxygen deficiency be the cause of the excess.

In view of Huckabee's (12) findings, the lack of time correlation between oxygen debt, recovery of muscle glycogen, and removal of blood lactate (3) is not surprising. As Fig. 1 clearly shows, all groups of trout, despite differences in oxygen debt, established a steady respiration rate similar to unexercised fish by 4–6 hours after fatigue. Levels of muscle glycogen and lactic acid did not return to normal for much longer. This probably means that, as in mammals, all the accumulated lactate need not be oxidized when the oxygen debt has disappeared, but only that it bear a certain relationship to pyruvate, so that metabolic oxidations can return to normal rates.

On the basis of earlier work (14, 3) it can be assumed safely that under the conditions of exercise in this study, greater utilization of glycogen entails greater lactate production. Assuming that, during strenuous swimming, glycogen in muscle is completely hydrolyzed to triose acid, the amount of glycogen used should equal the amount of lactate produced—about 125 mg% in the trained trout and 80 mg% in untrained ones. These values are in close agreement with the highest levels of blood lactate reported by Black *et al.* (2) for yearlings of the same species. The production of more fixed acid by trained trout might imply the presence of a more adequate means of buffering.

In mammals, haemoglobin is directly or indirectly involved in buffering some 70% of the carbon dioxide produced during muscular exertion. Fixed acids are initially buffered by bicarbonate ($BHCO_3$), but the acid product (H_2CO_3) is buffered by haemoglobin. In addition, Anthony (1) has recently pointed out that as a respiratory pigment, in goldfish at any rate, haemoglobin is a reserve material not required for *resting* metabolism. In consequence, the data here are presented on the following assumption: In trout, the primary function of haemoglobin is to act as a buffer. Its respiratory function is of a secondary nature during resting metabolism, but may assume greater importance during extreme conditions, as, for example, during those encountered in muscular fatigue or in oxygen-poor waters (17).

Greater buffering and oxygen-carrying capacity thus are the obvious advantages of high haemoglobin concentrations to the trained fish. This is especially important if the respiratory role of the pigment is more significant during severe periods when removal of fixed acid is the pressing problem. The second advantage could also explain how the trained trout, despite a larger oxygen debt, could repay it as quickly as the untrained fish.

With the above information, it is now possible to construct a tentative *modus operandi.*

Two factors, (*a*) mobile energy reserves and (*b*) acid metabolites, hence tissue buffers, to a large extent determine the capacity of fish for short bursts of swimming. The fuel reserves preclude exhaustion of the muscle; the buffers ensure a large utilization of the fuel by virtue of their resistance to excess lactic acid. Excess lactic acid means oxygen deficiency; the greater the excess, the larger the oxygen debt to be repaid. In theory, either (*a*) or (*b*) could be limiting. However, in untrained trout, depletion of glycogen cannot be the limiting factor to activity since only 40% of it is used by the time the fish is exhausted; in view of Black's extensive studies, lactic acid is the probable inhibitor. It is not likely that in the trained trout glycogen becomes limiting since even here only some 70% of the available supply is used. Consequently, *the difference in stamina between trained and untrained trout becomes primarily an expression of the different amounts of fuel that they are able to burn.*

It should be added that this study has dealt with a very special aspect of fish performance—that which is limited by the need to repay an oxygen debt. Sustained swimming is another matter (8). However, Brett *et al.* (6) have shown that training increases the capacity for more-prolonged swimming as well. In addition, metabolic alterations which are responsible for oxygen debt formation in exercise are essentially similar at all grades of exertion and probably also in exertion too slight to produce an oxygen debt detectable by normal respiratory methods (12). Presumably, then, the metabolic advantages seen in short swimming bursts would serve a similar function in maintained performance.

Periodically, in this paper, the "fitness" of wild stock *S. clarki* has been compared with that of the hatchery-reared rainbow trout. On the basis of these data, it is probable that the swimming capacity of wild trout is superior to that of hatchery fish. Phillips *et al.* (16) support this conclusion, and some recent measurements (19) show that wild trout have greater stamina than hatchery fish over a fairly wide size range. Miller (13) observed lower mortality in plantings of wild trout than in hatchery stock, and, perhaps more to this paper's point, training trout in seminatural conditions reduced the mortality after planting. This adds empirical weight to the survival-value of training.

Acknowledgments

This study was first planned with the late Dr. R. B. Miller of the University of Alberta, and was completed subsequent to his death. An expansion of it, under the supervision of Dr. F. R. Hayes, was submitted to Dalhousie University in partial fulfillment of M.Sc. requirements. It is a pleasure to acknowledge my indebtedness to both of these men.

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LIFE HISTORIES OF SOME COMMON SPIDERS FROM TREES AND SHRUBS IN NOVA SCOTIA[1]

C. D. DONDALE

Abstract

At Kentville, Nova Scotia, *Araniella displicata* (Hentz), *Philodromus rufus* Walckenaer, *Philodromus cespiticolis* Walckenaer, *Paraphidippus marginatus* (Walckenaer), and *Metaphidippus protervus* (Walckenaer) attained reproductive maturity in May and June. Eggs were laid in late June and early July. The young of *A. displicata*, *P. rufus*, and *M. protervus* began to appear in late July, while those of the remaining species began in early August. In *A. displicata*, *P. rufus*, *P. cespiticolis*, and *P. marginatus* there were two generations containing immature spiders at all times of the year, as indicated by frequency distributions of carapace width. It was concluded that these species are biennial in development. *M. protervus*, with only one generation containing immatures in any season, is annual.

Introduction

The life histories of very few spiders are known in sufficient detail to provide a sound basis for ecological work with these animals. One reason for this is the lack of reliable rearing methods by which life histories might be determined directly from insectary cultures. Another reason is the inconstancy in the number of developmental instars. As shown by Bonnet (1) and others, the number varies even among the offspring of a single female when reared under apparently identical conditions, and some individuals differ from others at maturity by as many as four instars. As Bonnet has also shown, there is a direct relationship between the number of instars and the size of the body, and the size will vary considerably among spiders of the same species and the same age. There is also variability in size, within a given generation, because of a time spread in hatching. A combination of these and other sources of variation results in a confusing array of spiders of different sizes in the field at any one time of year. It is the purpose of this paper to show that, in spite of these differences in size, the life history of a species can be reconstructed by analysis of size-groups in the field.

This study of the life histories of five species of tree and shrub spiders was conducted at Kentville, Nova Scotia, from 1958 to 1960. The species studied were an orb weaver, *Araniella displicata* (Hentz), two crab spiders, *Philodromus rufus* Walckenaer and *Philodromus cespiticolis* Walckenaer, and two jumping spiders, *Paraphidippus marginatus* (Walckenaer) and *Metaphidippus protervus* (Walckenaer). They were selected primarily because of their local abundance and ease of collection. Their life histories are set forth in broad outline only, the main features being hatching time, overwintering stages, approximate rates of development, and total life span. Other aspects of their biology, such as duration of the various stages in individual spiders and the effects of food, weather, and similar factors upon them, are left for future studies.

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Procedure

For all five species a single generation is added to the population annually. This assumption is based on the work of Bonnet (1, 2), Jones (7), Kaston (8), and others, whose observations are in agreement concerning the principal seasonal relationships among the spiders of trees and shrubs in the North Temperate Zone. If a species is spring-maturing, it overwinters in the immature state, and adults appear and mate in the spring; eggs are laid soon after, and the adults gradually disappear during the summer and autumn. The offspring of these adults spend the winter partly grown. The males are generally short-lived, and the mating period covers only a few weeks. The females receive enough sperm in their seminal receptacles to fertilize all of the oöcytes that they might produce. If the species is autumn-maturing, maturity and reproduction occur during late summer and early autumn. The adults die at the onset of winter, and their offspring continue growing during the succeeding spring and summer. From these observations it has been almost unanimously concluded that both spring and autumn species have a developmental time of about one year. As this paper shows, however, the possibility of biennialism should not be overlooked, because species requiring two years to reach maturity could include a certain proportion of reproducing individuals each year in the same way as do biennial plants.

Because a given generation hatches during July and August of a single year, there is a minimum of 3 to 5 months' growth between successive generations living at the same time in a particular locality. It was therefore hypothesized that generations are distinguishable on the basis of body size. The method chosen to test this was the collection and interpretation of samples of spiders at half-monthly intervals during three successive growing seasons. The establishment of distinct size groups then permitted the reconstruction of life histories of the five species. Insectary rearing provided supporting evidence at several points.

It was first necessary to select a suitable indicator of body size, preferably one that varied little between spiders of the same species and at the same stage of development. Volumetric and weight characters were not considered suitable because it was felt that they would be too much affected by day-to-day variation in food intake. Several linear characters were therefore assayed for variability in newly hatched and adult specimens of the five species. The characters measured were total length of body, length and width of carapace, length of femur II, and length and width of the ocular quadrangle. Measurements on strongly sclerotized parts of the body, such as the carapace and legs, were generally less variable than total length of body, which involved the abdomen (Table I). This is because all growth in the hard parts occurs at the time of ecdysis, while the abdomen, which has a soft, distensible integument, expands and contracts according to the amount of food in the gut. In the female spiders that are mature or nearly so, the abdomen is subject to additional changes in volume as the oöcytes mature and leave the body. None of the characters was consistently lowest in variability. The measurements of carapace and of ocular quadrangle show variation partly because of the difficulty in orienting the body in consistent fashion for measurement. In the two salticid

TABLE I

Variability of six linear characters in newly hatched and adult spiders at Kentville, Nova Scotia, based on samples of 25 individuals

($\bar{x}$ = sample mean, s = standard deviation, C.V. = coefficient of variability)

	Total length		Carapace length		Carapace width	
	$\bar{x} \pm s$ (mm)	C.V.	$\bar{x} \pm s$ (mm)	C.V.	$\bar{x} \pm s$ (mm)	C.V.
Araniella displicata						
Newly hatched	1.39±.11	7.6	0.63±.04	6.5	0.55±.03	5.1
Males	3.90±.30	7.8	1.91±.15	7.9	1.74±.10	5.4
Females	4.97±.67	13.4	2.09±.18	8.7	1.71±.14	8.1
Philodromus rufus						
Newly hatched	1.25±.13	10.2	0.54±.03	5.5	0.52±.02	4.5
Males	2.98±.22	7.5	1.36±.07	5.2	1.33±.07	4.9
Females	3.50±.35	9.8	1.40±.08	5.5	1.36±.08	6.0
Philodromus cespiticolis						
Newly hatched	1.15±.09	7.7	0.54±.03	4.7	0.59±.04	6.8
Males	3.98±.32	8.1	1.89±.11	5.8	1.96±.09	4.5
Females	4.79±.43	8.9	1.93±.09	4.7	1.93±.13	6.8
Paraphidippus marginatus						
Newly hatched	2.15±.13	6.0	1.05±.06	5.2	0.76±.05	6.6
Males	5.33±.62	11.6	2.6[illegible]±.29	11.0	2.14±.31	14.4
Females	6.29±.46	7.3	2.7[illegible]±.19	6.8	2.18±.21	9.4
Metaphidippus protervus						
Newly hatched	1.85±.17	9.3	0.95±.04	3.7	0.69±.03	4.1
Males	3.57±.25	6.9	1.74±.09	5.3	1.31±.07	5.4
Females	4.76±.44	9.3	1.89±.10	5.1	1.38±.07	5.2

TABLE I (*Concluded*)

	Femur II length		Ocular quadrangle length		Ocular quadrangle width	
	$\bar{x} \pm s$ (mm)	C.V.	$\bar{x} \pm s$ (mm)	C.V.	$\bar{x} \pm s$ (mm)	C.V.
Araniella displicata						
Newly hatched	0.40 ± .03	8.4	0.14 ± .01	9.3	0.18 ± .02	8.1
Males	1.79 ± .10	5.5	0.23 ± .03	14.4	0.27 ± .02	7.1
Females	1.63 ± .16	9.6	0.25 ± .04	15.4	0.30 ± .04	11.8
Philodromus rufus						
Newly hatched	0.45 ± .02	5.3	0.14 ± .01	10.1	0.21 ± .01	5.8
Males	2.01 ± .13	6.2	0.23 ± .02	8.9	0.38 ± .02	4.6
Females	1.84 ± .16	8.7	0.24 ± .02	8.5	0.42 ± .02	5.1
Philodromus cespiticolis						
Newly hatched	0.43 ± .05	12.0	0.13 ± .01	9.2	0.18 ± .01	5.0
Males	2.87 ± .20	6.8	0.27 ± .03	10.6	0.38 ± .02	4.0
Females	2.43 ± .15	6.3	0.30 ± .03	8.9	0.43 ± .02	4.1
Paraphidippus marginatus						
Newly hatched	0.42 ± .03	6.7	0.53 ± .03	5.7	0.76 ± .04	5.3
Males	1.35 ± .18	13.5	1.26 ± .12	9.7	1.65 ± .16	9.5
Females	1.33 ± .11	8.1	1.24 ± .09	7.2	1.81 ± .11	6.1
Metaphidippus protervus						
Newly hatched	0.39 ± .03	6.5	0.48 ± .04	7.3	0.67 ± .03	4.0
Males	0.84 ± .06	7.0	0.83 ± .04	5.3	1.08 ± .05	4.8
Females	0.88 ± .04	5.0	0.89 ± .05	5.1	1.21 ± .05	4.3

species, males were more variable in all characters than females, while in the other species the reverse was usually true. Newly hatched spiders were usually less variable than adults of the same species. The character eventually selected as a size indicator was the width of the carapace. It was uniformly the least variable character in *A. displicata*, usually second or third least in *M. protervus* and the crab spiders, and greatest in variability in *P. marginatus*. The last-named species was, however, quite variable in nearly every character. The carapace of spiders has regular and sharp margins, and measurement of its width requires little if any lifting away of other body parts.

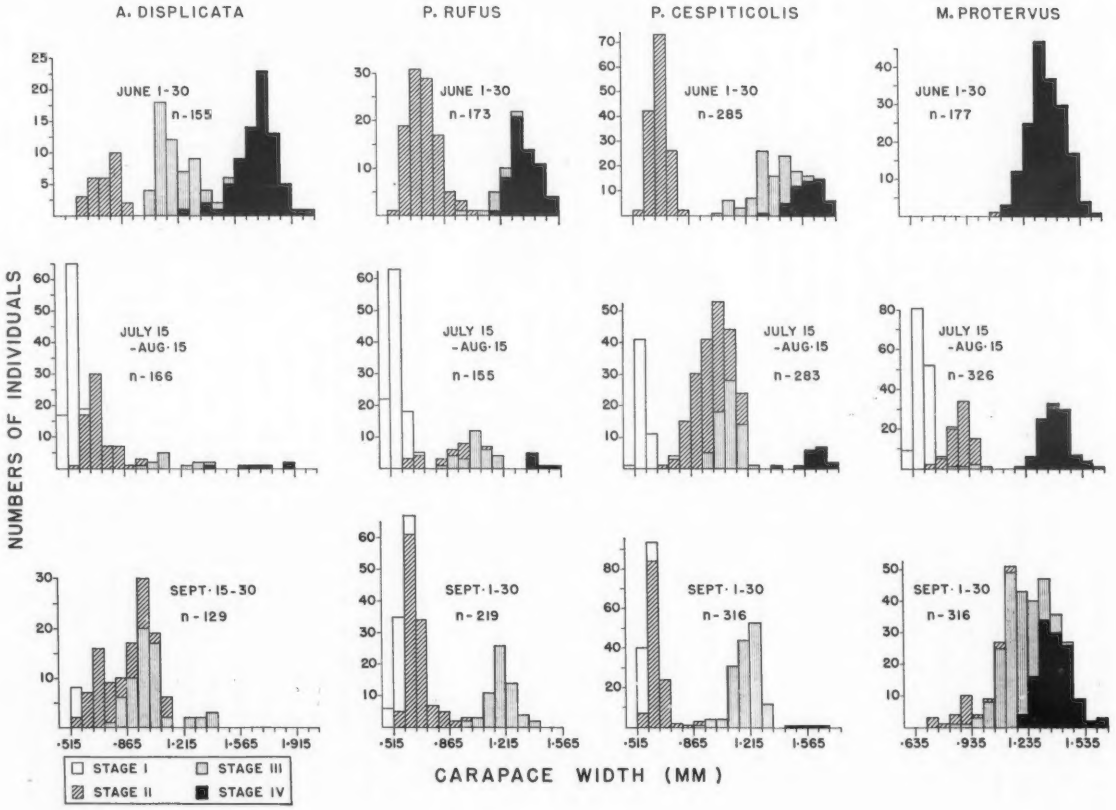
The width of carapace in adult spiders continued to be stable with the passage of time. In samples of 20 to 30 individuals, adults collected soon after their final ecdysis did not differ significantly from those collected later in the summer or autumn, the sexes being measured separately. Autumn males of *M. protervus* were similar in size to reproducing males collected in the spring. When males were compared with females of the same species, none of the differences were significant, although that in *M. protervus* approached the 5% level.

Measurements of carapace width were made in the half-monthly collections of the five species of spiders under study. The numbers of individuals that fell into various size-classes were recorded for each collection, but only the data from three periods are reported. The first period occurred in June, when emergence from winter quarters was complete and most of the spiders had resumed growth. The second period was in late July and early August, when the new generation was making its appearance. The third was in September, when the spiders had essentially completed their growth for the season, and a few of them were beginning to move from tree foliage to overwintering places.

Results

Carapace widths and stages of development are shown for the three above-mentioned periods in Figs. 1 and 2. In general the first adults were observed in June, and mating began at about the same time. Egg-laying commenced 10 to 15 days later, and the first offspring appeared at the end of July (*A. displicata*, *P. rufus*, *M. protervus*) or early in August (*P. cespiticolis*, *P. marginatus*). The parents of these offspring dwindled in numbers throughout the summer, only senescent females being found after mid-August. These parents undoubtedly comprised a generation, because they form a distinct size-group in the histograms (Figs. 1 and 2). The June histograms show these adults developing from overwintered stage-III individuals (subadults), a process observed by bringing many of the large immature spiders into the insectary and feeding them on *Drosophila* until mature. Newly hatched individuals were also reared for one season in this way, and these were found to grow quite uniformly, the sizes and stages of development paralleling the size-groups found in field samples. Further rearing was prevented by the death of the cultures during the winter.

In *P. rufus* and *P. cespiticolis* (Fig. 1) the population in June was composed of a large group of overwintered stage-II individuals and a smaller group of adults and subadults. In late July and early August, when maturation


A. DISPLICATA
P. RUFUS
P. CESPITICOLIS
M. PROTERVUS
JUNE 1-30
n-155
JUNE 1-30
n-173
JUNE 1-30
n-285
JUNE 1-30
n-177
JULY 15
-AUG·15
n-166
JULY 15
-AUG·15
n-155
JULY 15
-AUG·15
n-283
JULY 15
-AUG·15
n-326
SEPT·15-30
n-129
SEPT·1-30
n-219
SEPT·1-30
n-316
SEPT·1-30
n-316
NUMBERS OF INDIVIDUALS
·515 ·865 1·215 1·565 1·915
·515 ·865 1·215 1·565
·515 ·865 1·215 1·565
·635 ·935 1·235 1·535
STAGE I
STAGE II
STAGE III
STAGE IV
CARAPACE WIDTH (MM)

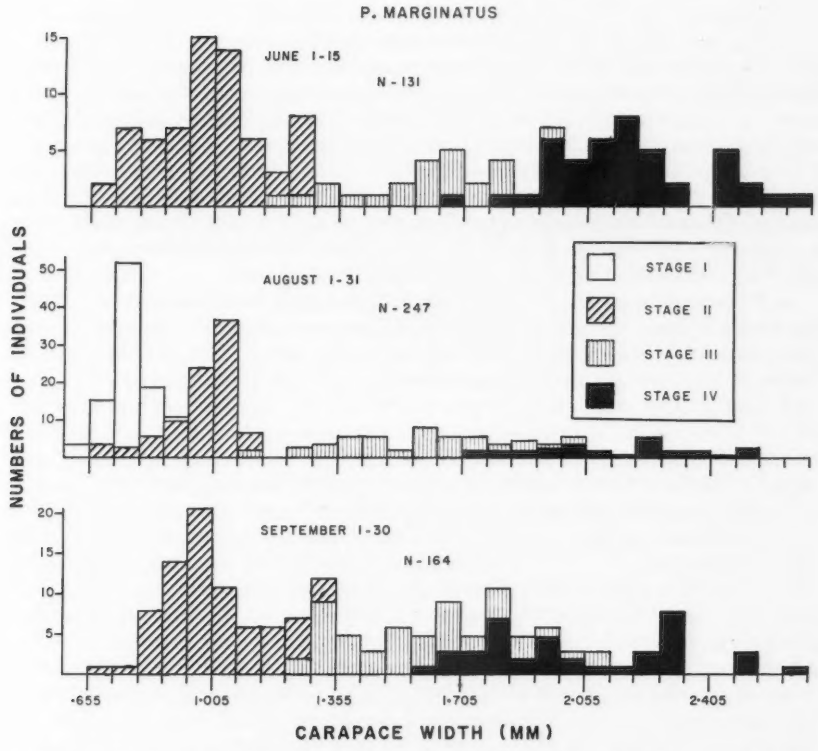


FIG. 2. Frequency distributions of carapace widths in *Paraphidippus marginatus* (Walckenaer) at Kentville, Nova Scotia, from accumulated collections of 1958–60. Stage I, newly hatched; stage II, intermediate; stage III, subadult; stage IV, adult.

and mating among members of the larger group had ended, these adults began to disappear and were replaced by a new generation of stage-I individuals. The stage-II individuals of June, however, were still present, developing into stage III as shown by the appearance of genital swellings under the palpal skin of males and in the epigynal area of females. The September samples contained few or none of the parent generation, although in both species occasional females, very shrunken and weak, have been taken in general collections as late as November. The new generation had largely developed to stage II by September, and the half-grown group of July and August had become nearly mature.

FIG. 1. Frequency distributions of carapace widths in *Araniella displicata* (Hentz), *Philodromus rufus* Walckenaer, *Philodromus cespiticolis* Walckenaer, and *Metaphidippus protervus* (Walckenaer) at Kentville, Nova Scotia, from accumulated collections of 1958–60. Stage I, newly hatched; stage II, intermediate; stage III, subadult; stage IV, adult.

The seasonal picture of the web-builder *A. displicata* is essentially the same as for the two species of *Philodromus*, but minor differences appear (Fig. 1). The subadults and adults of June were quite different in carapace width. Subadults of various sizes that were brought to the insectary for rearing, however, all reached maturity before mid-July. In early August few adults remained, and the new generation had already progressed well into stage II. The small group in stage III had developed from the stage II individuals of June. In September no adults remained, and many of the new generation were in stage III, while others remained in stage I or II. A few large subadults with a carapace width in excess of 1.20 mm were the only representatives of the generation that would become mature the following spring.

In *P. marginatus* (Fig. 2) the pattern is essentially like that in *A. displicata* and in *Philodromus*, but it is obscured by greater variability. Two generations were present in June, the smaller becoming half-grown by early August and subadult by September, the larger becoming mature and laying eggs in June and July. Adults persisted throughout the summer and autumn, a situation which, particularly for males, appears to be unusual. It is not known whether or not these postreproductive adults live through the winter at Kentville, but as yet none have been found in winter collections. Those spiders that hatched in August were all in stage II by September.

The seasonal development of *M. protervus* (Fig. 1) was quite different from that of the other species. In June the entire population was mature or nearly so, and mating and egg-laying were both nearly complete by the end of that month. In late July and early August the males had disappeared, leaving only old females and their fast-growing offspring. Some individuals of the new generation had reached stage III by mid-August. In September the population was represented as a single large group in the histogram, and was observed to contain a few of the parent females, but was composed mainly of subadult females and lightly pigmented males. These subadult and newly matured spiders obviously belonged to the new generation. Samples taken toward the end of September contained no further individuals in stage II, and it was concluded that *M. protervus* spends the winter entirely as adult males and subadult females.

From the seasonal distribution of body sizes and of stages of development it is seen that four of the five species have two generations in the field during most of the year, both generations containing immature individuals. Another generation is present during the time that parents and their offspring remain together in the field, a period extending from late July to September or even until the advent of freezing temperatures, according to species. The fifth species, *M. protervus*, has a simpler developmental pattern: a single generation of immature spiders exists in autumn, winter, and early spring. The maximum number of generations present at any time is two, comprising one of parents and one of their offspring.

The number of generations in the field in spring and autumn reflects the length of the maturation time in spiders. Gertsch (5) states the seasonal relationships of annual species as follows: "Thus, in the spring and early summer we have the males and females of the spring fauna, ...", and "after the killing

frosts of November . . . the growing young of the spring spiders have attained nearly their full development." This pattern of a single generation for most of the year fits precisely *M. protervus*, and this species is therefore considered to have a 1-year developmental time. Females, of course, lived a number of months after becoming mature.

The more complex patterns presented by the other four spiders remain to be explained. Palmgren (10) has given sufficient details of the life history of the European pisaurid *Dolomedes fimbriatus* (Clerck) in Finland to make it quite certain that the life histories of these Nova Scotian species are the same as that of *D. fimbriatus*. In the latter species a certain proportion of the population matures, mates, and produces offspring each summer. The young spend their first winter in a very early stage of development, attain about half their total growth during the second summer, overwinter a second time in a nearly mature state, and become mature early in the third summer. There would thus be two immature generations in the field in autumn, winter, and spring, and the species is biennial in life history. By analogy, the four species *A. displicata*, *P. rufus*, *P. cespiticolis*, and *P. marginatus* are also biennial.

Since the four species found to be biennials are all rather widespread and abundant, it seemed possible that other species could also be biennial but had not been recognized as such. Accordingly several of these tree- and shrub-dwelling spiders of eastern Canada were collected in autumn to determine the number of generations in the field at that season. The following were found to have two distinct size-groups, or generations, which indicates that the species are biennials:

Theridiidae: *Theridion differens* Emerton
Theridion murarium Emerton
Erigonidae: *Dismodicus decemoculatus* (Emerton)
Thomisidae: *Misumena vatia* (Clerck)
Misumenops asperatus (Hentz)
Xysticus punctatus Keyserling
Philodromus praelustris Keyserling
Philodromus placidus Banks
Philodromus vulgaris (Hentz)

Other species appeared to exist in two size-groups in autumn, but too few specimens were available to permit their definite inclusion in the list. In any case, enough species have been cited to show that biennialism is fairly prevalent among tree and shrub spiders of the North Temperate Zone.

The time required for *A. displicata*, *P. rufus*, *P. cespiticolis*, and *P. marginatus* to reach reproductive maturity was 22–23 months, and their total life span was 24–28 months. In *M. protervus*, reproductive maturity was attained in 11 months, and the life span was 12–16 months.

A modification in the *Araniella* and *Paraphidippus* patterns was observed in 1960. In *Araniella*, many of the spiderlings that hatched in July were showing distinct genital swellings by the end of September, i.e., they had progressed from stage I to stage III during their first growing season. If two or more such years of rapid growth should occur in succession, the species could become, at least temporarily, annual in development. The summer of 1960 was an unusually warm one at Kentville. In August and September of that year the daily mean temperatures were 67.3° and 59.2° F, respectively, as compared with

means for the preceding 10 years of 64.3° and 57.1° F. In more southerly parts of the range of *Araniella*, it is possible that developmental time is normally shorter than at Kentville, but the problem requires further investigation.

In *Paraphidippus* many undersized males that were not yet fully pigmented appeared in the samples. These males seemed to belong to the generation that hatched in 1959, and had therefore reached anatomical maturity in about 14 months instead of the usual 22–23 months. Some of the newly hatched individuals of 1960 also had reached stage III by late September, as in *Araniella*. In Connecticut Kaston (8) found *P. marginatus* overwintering as mature males and subadult females, a situation which seems to have occurred in 1960 at Kentville owing to unusual summer temperatures.

An attempt was made to detect seasonal trends in growth rates in the five species. Mean carapace width was plotted at half-monthly intervals from the time of hatching until maturity was reached. Although the curves showed successive increases in size during each growing season, the mean increases were not significant owing to the level of variation within samples. More precise observations on individual spiders in captivity are needed before valid inferences about growth rates can be drawn.

Discussion

The spiders whose life histories are described here are representatives of a large group in which reproductive maturity is always reached in the spring. To the same group also belong numerous other thomisids and salticids, as well as large segments of other families such as Theridiidae, Clubionidae, and Lycosidae (5). Included are spiders that attain maturity within one year (*M. protervus*, *Pardosa moesta* Banks), and others that require two years (*A. displicata*, *P. rufus*, *P. cespiticolis*, *P. marginatus*, *Dolomedes fimbriatus*). The principal modifications in length of developmental time in spring species are the prior attainment of anatomical maturity in one or both sexes, and a shortening due to a more favorable growing season. In *M. protervus*, males reach full size in the autumn of the year in which they hatch, but do not mate until spring, when the females molt for the last time. In *Trochosa pratensis* (Emerton), a biennial wolf spider, both sexes experience their final molt in the second autumn of life, but mating and egg-laying occur the following spring (6). Various species of *Geolycosa* are also biennial (9, 12). Here both the final molt and mating take place in the second autumn of life. The males die at the onset of cold weather, and the females lay their eggs the following spring.

The effects of seasonal influences such as temperature, humidity, and photoperiod on spider development are little understood. *A. displicata* and *P. marginatus*, which are normally biennial in eastern Canada, are believed to have a shorter developmental time when the growing season is an unusually warm one. There is evidence that this more rapid type of development is normal for these species in more southerly latitudes. In the same way *Dolomedes* is biennial in northern Europe (10), but is believed to be partly annual at Toulouse, France, and to be completely annual in northern Italy (1). It has been speculated that spiders whose ranges include both temperate and arctic zones of the world might require much longer to reach maturity in the far

north than in southern parts of their ranges (3, 5). On the other hand, it is quite possible that the long days and abundance of dipterous food during the arctic summer permit spiders to develop in the same number of seasons as they do in southern Canada. This, too, invites investigation.

As opposed to the spring-maturing spiders there is a second large group in which reproductive maturity is confined to late summer and autumn. These may be considered short-day species, and they are found in such genera as *Linyphia*, *Araneus*, *Zygiella*, *Argiope*, and *Agelenopsis*. Observations on several species in the group indicate a basic developmental time of one year (1, 4, 11), but there is evidence for a lengthening in at least two species, namely, *Araneus diadematus* Clerck and *A. circe* (Sav. & Aud.). According to Wiehle (13) these species require nearly two summers to mature in the northern parts of their ranges.

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THE RELATION BETWEEN NEUROSECRETION AND CELL DIFFERENTIATION IN THE OVOTESTIS OF SLUGS (GASTEROPODA: PULMONATA)[1]

D. Pelluet and Nancy Jane Lane

Abstract

Cytodifferentiation in the germinal epithelium of the ovotestis of slugs is approached experimentally. The tentacles of the slugs were cut off and the animals maintained for different lengths of time before the ovotestis was examined. In both species of slug used, *Arion subfuscus* and *Arion ater*, the experimental animals showed a noticeable increase in the number of eggs, when compared with the control. Solutions of brain homogenate and tentacle homogenate were then injected separately into intact animals. The brain solution apparently stimulated the production of eggs, the numbers being nearly equal to that produced by cutting the tentacles. The tentacle solution injected into the animals produced no increase in the number of eggs, and in young animals the normal growth of the ovotestis was inhibited. Cytological study of the brain and tentacles reveals neurosecretory cells and a close association with the blood system of the animal, suggesting a basis for a hormonal system. In this case, a theoretical concept of a dual hormonal control is put forward, to explain the production of male cells first by the tentacle hormone, followed by egg production under the control of a brain hormone.

Introduction

The problem of the differentiation of the cells of the ovotestis in slugs has been under consideration for a number of years, during which it was found that environmental factors such as temperature (3) and light (10) bring about disorganization of the whole ovotestis to a marked degree, although there was no tendency for the germinal epithelium to differentiate in the direction of one sex, rather than of both sexes in the normal way. However, in 1952, Bridgeford and Pelluet (3) suggested that the normal differentiation might be regulated by a chemical substance, perhaps a hormone, whose action might be interfered with by exposure to the low temperatures. This idea suggested to the authors that perhaps a vertebrate hormone ("Synapoidin") might have some effect on the cell differentiation. On injection of the "synapoidin" an increase in the number of eggs in each follicle was apparent. This result suggested an experimental approach to the problem of the relation between neurosecretion and cell differentiation, which forms the subject of this present work.

The production of hormones in invertebrates is now a well-established fact and the site of their origin lies in the central nervous system and its associated structures. The literature on the production of hormones by neurosecretory cells in invertebrates is now voluminous and much of it is to be found in the extensive review of Scharrer and Scharrer (14) which covers the work, up to 1954, on flatworms, annelid worms, crustacea, insects, and molluscs.

The work on the neuroendocrine system of arthropods, on which more has been done than on any other invertebrate group, is the subject of a review

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Contribution from the Department of Zoology, Dalhousie University, Halifax, Nova Scotia.

by Scheer (15) in an article containing over six hundred references. The problem of the influence of hormones and chemical substances on the differentiation of individual cells has been analyzed in the epidermis of insects by Wigglesworth (18), whose earlier publications laid the foundations of our present knowledge of insect endocrinology.

The ovotestis of slugs presents a somewhat different problem in cytodifferentiation from that of animals with separate sexes, or in hermaphroditic animals like *Lumbricus* in which the testes and ovaries are developed independently. In slugs, the germinal epithelium lining the gonad gives rise to nurse cells, egg cells, and spermatogonial cells lying in close contact with each other in the same follicle of the ovotestis of the mature animal.

Material

The experimental work was carried out on the genus *Arion*, using two species, *A. subfuscus* (Drapanaud), a native form, and *A. ater*, imported from the Pacific coast. Specimens of the genus *Limax* were used for making histological preparations of the brain, as they are larger than *Arion*, but otherwise the structure is alike in the two genera.

The method of maintaining the animals in the laboratory has been described in an earlier paper (13).

Experimental Methods

1. The tips of the optic tentacles were cut off, removing the eye, optic and tentacular nerves, sensory digitate ganglion, and the glandular collar cells. The animals were put back in the containers for different periods of time, after which measurements of the sizes of body and ovotestis were made and then their wet weights taken separately. The experimental results were expressed as a ratio,

$$\frac{\text{ovotestis weight}}{\text{body weight}} \times 100,$$

following Wells and Wells (17), who found this method satisfactory for expressing changes in gonad weight relative to body weight in octopuses. These measurements are contained in Tables I and II.

2. The ovotestis of these same animals was then examined in the following way: The ovotestis was removed, placed in slug saline to which had been added a small amount of trypsin, in order to loosen the tough connective tissue surrounding the acini of the ovotestis (12). After about 15 minutes, the whole ovotestis was transferred to a solution of approximately 1% methyl green for 10 minutes, after which it was put in a bath of cedarwood oil for clearing. This method resulted in the eggs showing up well with a clearly recognizable nucleus and prominent nucleolus, while the sperms were stained green. The ovotestis was placed on a slide and flattened by pressure on the cover slip. The eggs were counted with an ocular counting grid and a hand Veedor-Root counter. Counts were made carefully once in successful preparations. If the clearing had not been quite complete, an average of two or three counts was taken.

3. Extracts of brain tissue and of tentacle tissue were made separately, using 35 animals. The dissections and homogenization of the tissues were carried out in a cold room at 4° C. The tissues were frozen immediately by

immersion in a bath of isopentane and propane at about −190° C. The brains and tentacles were put into two separate bowls and were cut into small pieces while still frozen. As the amount of material was too small to be used in a hand homogenizer-tube, the frozen pieces were placed in a small mortar with silica sand, and ground with a pestle, in isotonic saline (pH 8.5) yielding a total of 3 ml. Both the solutions were well stirred, and the solutions centrifuged at about 4000 revolutions per minute for 10 minutes. This method produced a closely packed residue and a clear supernatant for injection. The injections were made into the body cavity by means of a hypodermic needle. *Arion ater* was given 0.1 ml and *A. subfuscus*, 0.05 ml. The controls were injected with saline only. A second injection was given to all the animals 1 week later. The small number of controls seemed permissible in view of previous work (3) with injection of saline, which had no harmful effects on young animals, even after several injections.

4. The remainder of the solutions left after injection were examined chemically in an attempt to determine what, if any, type of hormone might be in the solutions. Tests for proteins, amino acids, peptide linkages, sterols, carbohydrates, and reducing sugars were carried out according to the technique of Cole (5).

Results of the Experiments

1. The relation between the body weight and the ovotestis weight in control and experimental animals is shown in Fig. 1.

2. In studies with animals with tentacles cut off (see Table I), young animals show marked variations in the size of the body and of the ovotestis. In order to reduce the figures to a common denominator, so that one could compare the number of eggs in small and large ovotestes, the wet weights of the slug and of its ovotestis were expressed as a ratio, (ovotestis weight/body weight)×100, and the resulting figure plotted against the number of eggs. The graphs in Figs. 2 and 3 show that the removal of the optic tentacles results in an increase in the number of eggs in comparison with that of the controls. The difference in histological appearance in the two sets of animals is illustrated in Figs. 9 and 10.

The acini of these experimental animals contained 4 or 5 eggs crowded together whereas normally there would be 1 to 1.5 eggs in each acinus (3). The removal of the tentacles resulted in a reduction in both volume and size of the gonad in young animals, indicating that the actual growth of the gonad had been affected (Fig. 4) in spite of the increase in the number of eggs. This reduction in gonad weight was at first sight unexpected, since there were more eggs in the experimental animals, which might have been expected to add to the weight of the gonad. It is probably due to the fact that the values were recorded as wet weights, and these would be greater for the larger normal ovotestis filled with masses of sperm than for the more compact egg-filled gonad of the experimental animals. With older animals this change in weight was not so noticeable, presumably because there was not such a marked difference in gonad size in larger animals.

3. After brain homogenate was injected into the body cavity of intact animals, they were left in the bowls for about 2 weeks before examination.

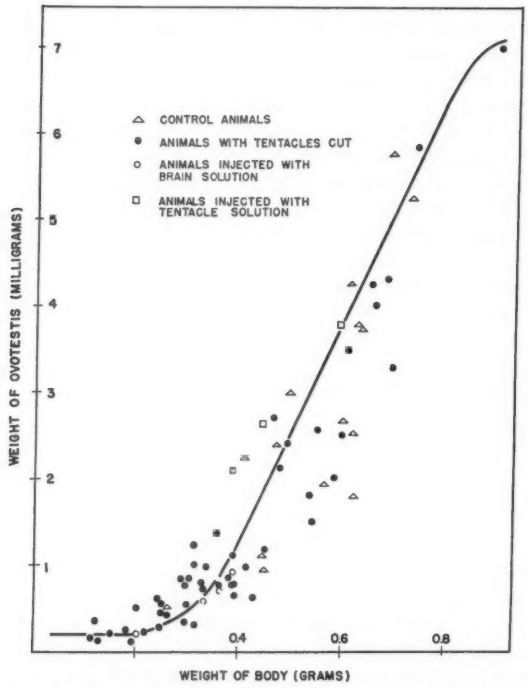

FIG. 1. Increase in ovotestis weight with increase in body weight in normal animals, animals with tentacles removed, injected.

The ovotestes were treated in the manner previously described and it was found that the number of eggs exceeded that of the controls. Table II contains the data for both species of *Arion*. The results are shown graphically for *A. subfuscus* in Fig. 5. The graph for *A. ater* is not included, since the results are similar. The results of injecting tentacle solution into slugs show that no more eggs are produced than in the control animal. This would be expected on the theory that the hormone of the tentacle either inhibits egg production until maturity or regulates the output of the brain hormone. Apparently extra doses of brain hormone can overcome this inhibition, as seen in these results.

4. The biochemical assay made on brain and tentacle homogenates gave similar results in general. Solubility tests for lipids, using petroleum ether and chloroform, fat, or sterol solvents, gave negative results. Much of the central nervous tissue of slugs was saline- or water-soluble and these solutions gave positive tests for the presence of amino acids, peptides, and proteins as well as for polysaccharides. From this general biochemical analysis one can state that the active substances producing the experimental results were protein or glycoprotein. From the experimental results, there exists a difference in

TABLE I

Results of cutting off the tentacles on the weight and number of eggs in the ovotestis

	No. of animals	No. of weeks after tentacle severance	Av. wt. of body $\pm\sigma_M$	Av. wt. of ovotestis $\pm\sigma_M$	Av. no. of eggs	Av. ratio of ovotestis wt. to body wt. $\times 100$ $\pm\sigma_M$
Arion subfuscus Control animals	36	0	0.445 ± 0.044	0.004 ± 0.0006	36	0.724 ± 0.104
Experimental animals—optic tentacles cut	50	3–4	0.373 ± 0.026	0.0013 ± 0.0002	150	0.277 ± 0.027
Arion ater Control animals	23	0	2.227 ± 0.408	0.009 ± 0.0008	240	0.309 ± 0.075
Experimental animals—optic tentacles cut	23	7–8	1.781 ± 0.285	0.003 ± 0.0012	547	0.115 ± 0.002

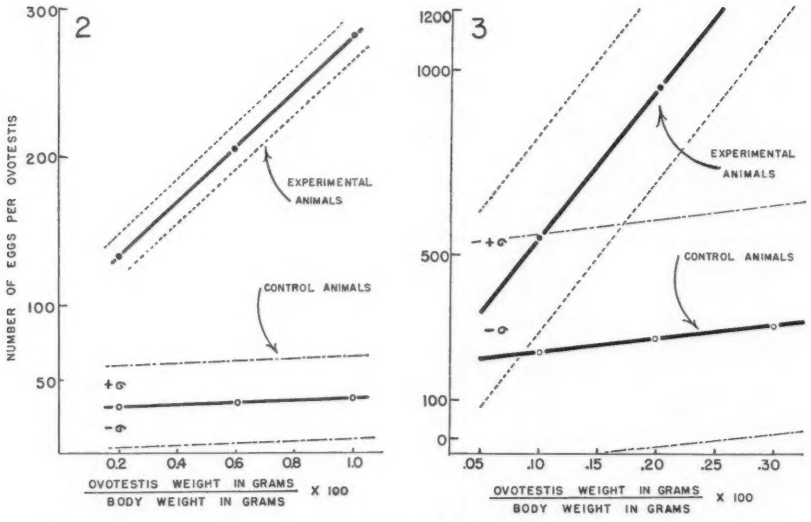

FIG. 2. Increase in number of eggs in *Arion subfuscus*, 3 to 4 weeks after cutting off the tentacles.

FIG. 3 Increase in number of eggs in *Arion ater*, 7 to 8 weeks after cutting off the tentacles.

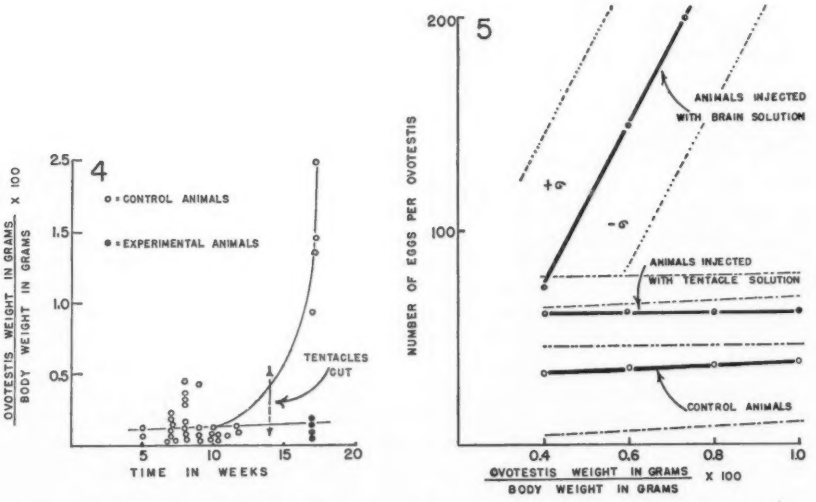

FIG. 4. Inhibition of growth of the ovotestis in *Arion subfuscus* following the removal of tentacles in young animals.

FIG. 5. Effect on number of eggs in ovotestis of injecting brain solution and tentacle solution into intact animals of *Arion subfuscus*.

TABLE II

Results of injection of brain and tentacle solutions

Animals and material injected	No. of animals used	No. of weeks after injection	Av. wt. of body $\pm\sigma_M$	Av. wt. of ovotestis $\pm\sigma_M$	Av. no. of eggs	Av. ratio of ovotestis wt. to body wt. $\times 100$ $\pm\sigma_M$
Arion subfuscus						
Saline solution	4	2	0.058 ± 2.284	0.005 ± 0.023	62	1.096 ± 5.886
Brain solution	10	2	0.359 ± 0.064	0.0008 ± 0.0002	153	0.227 ± 0.019
Tentacle solution	10	2	0.653 ± 0.546	0.002 ± 0.003	63	0.438 ± 0.681
Arion ater						
Saline solution	4	3	1.381 ± 0.249	0.001 ± 0.0001	130	0.087 ± 0.019
Brain solution	10	3–4	3.122 ± 0.883	0.003 ± 0.001	300	0.101 ± 0.055
Tentacle solution	10	3–4	2.351 ± 0.813	0.002 ± 0.0006	113	0.068 ± 0.021

action in these two hormones, which may lie in a slight chemical difference. It is regrettable that one is unable to prove that the difference in staining of the neurosecretory droplets in the brain and in the glandular collar cells is a true reflection of the biochemical difference between the two hormones (Table IV).

Histological and Cytological Methods

The brain was dissected out as quickly as possible and put in an appropriate fixative. The main purpose of this part of the work was to establish the presence and distribution of neurosecretory cells in the brain and tentacles, which might be responsible for the experimental results. In addition, the cytological details of the neurones were studied in order to get as much information as possible on the invertebrate neurone.

The *fixatives* used were: Helly, Bouin, Zenker, Feulgen, Regaud, formalin, Champy Kull, Cajal, and Baker's osmic acid (2). The *stains:* for general histology—haematoxylin–eosin and the paraldehyde fuchsin method of Gomori (8); cytological, neurosecretory material—Clark's procedure for the paraldehyde fuchsin method (4); mitochondria—Altmann's acid fuchsin – toluidine blue – aurantia (1); Nissl substance—toluidine blue (19); polysaccharides—periodic acid – Schiff reagent (PAS) (11); Golgi material—Baker's method (2); neurofibrillae—Cajal's silver impregnation (9); live material—Vital staining with neutral red, methylene blue, and Janus green.

Material was embedded in histowax, or double embedding with celloidin was used. Sections were cut at 5 μ, except after Cajal fixation, when the material was cut at 8 μ.

Cytological and Histological Structure of the Nervous System of Slugs

A. The Brain

The anatomy of the gasteropod brain has been well known for a long time. Simpson's description and drawing of the nervous system of *Limax maximus* (16) is typical of other genera, such as *Arion* and *Deroceras*, with only very minor variations. There is, however, a difference in Simpson's description of the arrangement of the tentacle and its nerve supply. We find, in agreement with Demal (6), that the optic nerve has its origin from the supraoesophageal ganglion, and throughout its length it is quite separate from the tentacular nerve (Figs. 6, 12). The tentacular nerve terminates in a large, bulbous digitate ganglion, while the smaller optic nerve enters and innervates the eye. In the genera mentioned above the cephalic artery has the same general distribution. The cephalic artery runs through the infraoesophageal ganglia within the ring of ganglia surrounding the oesophagus. The artery runs round the brain sending branches into the two optic tentacles which arise in the supraoesophageal ganglia. The general distribution of the cephalic artery is diagrammatically indicated in Fig. 6. Horizontal serial sections were made through the brain in order to determine the positions of the various types of cells in the ganglia before carrying out the cytological study. Composite drawings were made from these and are shown in Figs. 7 and 8. The distribution and size of the cellular components of the ganglia and tentacles are

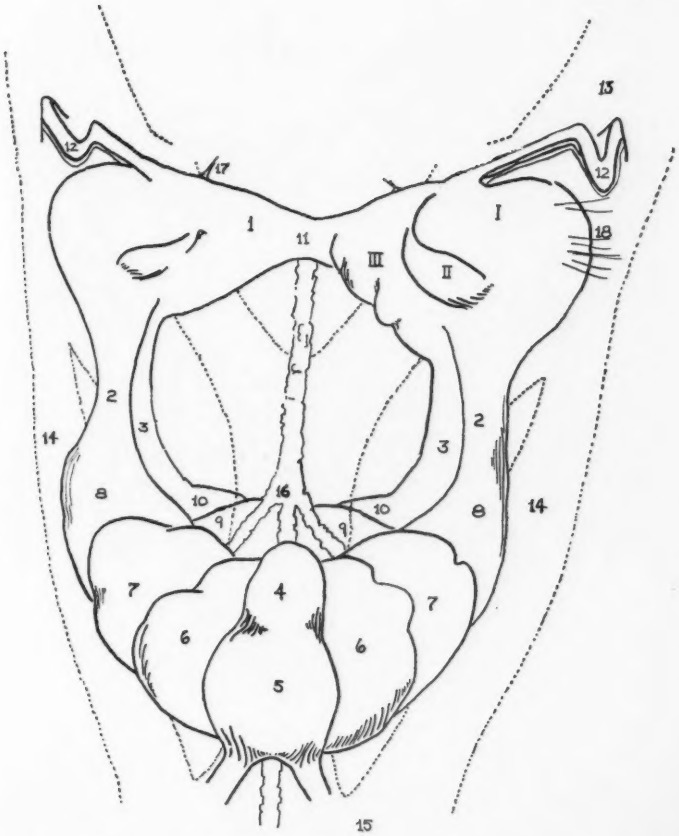

FIG. 6. Brain of *Limax maximus*, ×48.

Explanation: I, first lobe of the supraoesophageal ganglion, composed internally of central nerve fibers surrounded by a capsule of small cells; II, second ganglionic lobe of the supra, oesophageal ganglion; III, third ganglionic lobe, composed of four distinct smaller lobes; 1, supraoesophageal ganglion; 2, cerebral-pleural commisure, linking second and fourth lobes of the supra- and infra-oesophageal ganglia; 3, cerebral-pedal commisure, linking third and sixth lobes of the two ganglionic masses; 4, small lobe of the first ganglia pair of the infra-oesophageal ganglion; 5, large lobe of the first ganglia pair of the infraoesophageal ganglion; 6, second ganglia pair composing this part of the brain; 7, third ganglia pair; 8, fourth ganglia pair; 9, fifth ganglia pair; 10, sixth ganglia pair; 11, connective between the two lobes of the supraoesophageal ganglion; 12, optic and tentacular nerves entering the optic tentacle together; 13, optic tentacle; 14, muscle leading to optic tentacle; 15, point of union between buccal and tentacular retractor muscles in *Limax;* 16, cephalic artery at point of division; 17, buccal nerve leading to buccal ganglion; 18, nerves and connectives connecting tentacular muscle to brain.

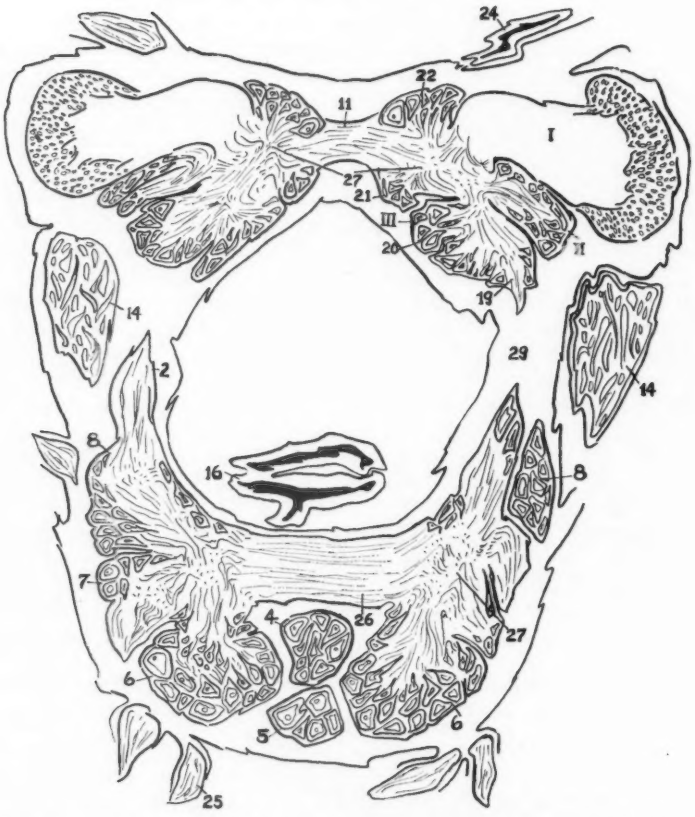

FIG. 7. Horizontal section of the brain showing histological arrangement, ×53.

Explanation: 1 to 18, same as for Fig. 6; 19, the first of the four smaller ganglionic masses comprising the third ganglionic lobe, it lies close against the second ganglionic lobe; 20, the second of these masses described in 19, lying against the first such mass; 21, the third of these masses, situated beside the second; 22, the fourth of these ganglionic masses, located more dorsally and near the first ganglionic lobe; 24, section of blood vessel leading to the optic tentacle; 25, nerves leaving suboesophageal ganglion for other parts of the body; 26, connectives between the third ganglion pair of the infraoesophageal ganglion; 27, area of horizontal, vertical, and lateral fiber tracts within ganglionic lobes, surrounded by neurones of varying sizes; 29, connective tissue surrounding and ensheathing the entire brain.

summarized in Table III. The main emphasis in this work was placed on establishing the presence or absence of neurosecretory elements in the nervous system. The term "neurosecretory" is used in the sense of Scharrer and Scharrer (14, p. 184). Neurosecretory droplets were found in neurones of all sizes with two exceptions—viz., the large cells at the most anterior and central end of the third lobe of the supraoesophageal ganglion and the cells of the most ventral and posterior part of the fifth lobes of the infraoesophageal ganglia.

FIG. 8. Horizontal section of the brain showing histological arrangement, ×53.

Explanation: 1 to 18, same as for Fig. 6; 19 to 22, 24 to 27, 29, same as for Fig. 7; 23, large peculiarly staining cells on surface of the fifth lobe of the infraoesophageal ganglion; 28, ventral surface of capsule of small cells of the first lobe of the supraoesophageal ganglion, no longer showing all of the central fibrous tract; 30, elliptical cross section of spheroidal otocyst, the organ of hearing; 31, small calcareous bodies of varying size within the otocyst, which oscillate against sensory hairs in the epithelial wall of this organ during life.

The nomenclature of the cytoplasmic components of the nerve cells is still very confused and the relationship between neurosecretory granules and other inclusions is far from being clearly established, so no attempt has been made here to interpret these obscure relationships.

The neurones of the brain are in general uni- or tri-polar, with a conspicuous single nucleus. The neurones range in size according to the position in the brain and a summary of the characteristics of these nerve cells is given in Table III. Attention may be directed to a somewhat different type of neurone surrounding the first lobe of the supraoesophageal ganglia (Fig. 6). The neurones are very small elliptical cells arranged many layers deep round the central fiber tract, with the innermost layers of cells sending axonal processes to this central mass, which is less fibrous than the other tracts. The

TABLE III

Data on neurone size, position, and nuclear staining in the brain and optic tentacles

Type of cell	Position	Size	Nuclear chromatin	Nucleolus
Fixed cells				
Neurones of the brain	Major component of both supra- and infra-oesophageal ganglia	Large 73×44μ Medium 53×32μ to 39×18μ Small 14×9μ	Diffuse granular appearance with clumps of darkly staining chromatin	Numerous—8 to 30 with fewer in the smaller cells + orange G + light green + pyronine
Cells of supraoesophageal ganglion	Form an external capsule around the lateral first lobes of the supraoesophageal ganglia	On the average 6×4μ	Intensely staining chromatin clumps with a thread-like appearance	Apparently none
Cells in fifth lobe of infra-oesophageal ganglion	Compose the anterior portion of the fifth lobe of the infra-oesophageal ganglia next to the cephalic artery	On the average 83×50μ	Extremely coarse granular appearance	1 to 3 very large nucleoli which stain heavily
Glandular collar cells of optic tentacle	Surround optic and tentacular nerves and blood vessels in optic tentacle at distal end	Range from about 56×21μ to 37×16μ	Granular chromatin with a thread-like appearance and many clumps producing a beaded effect	A single intensely staining large nucleolus
Live cells				
Neurones of the brain	Cell components of the brain	Large 115×125μ to 61×43μ Medium 32×29μ Small 18×8μ to 8×8μ	Almost indistinguishable	Different nucleoli visible with different levels of adjustment
Glandular collar cells of optic tentacle	Surround optic and tentacular nerves and blood vessels in optic tentacle	Range from about 67×29μ to 30×25μ	Clearly visible, situated at one end of the cell, structureless save for nucleolus	Acentric and spheroidal single nucleolus

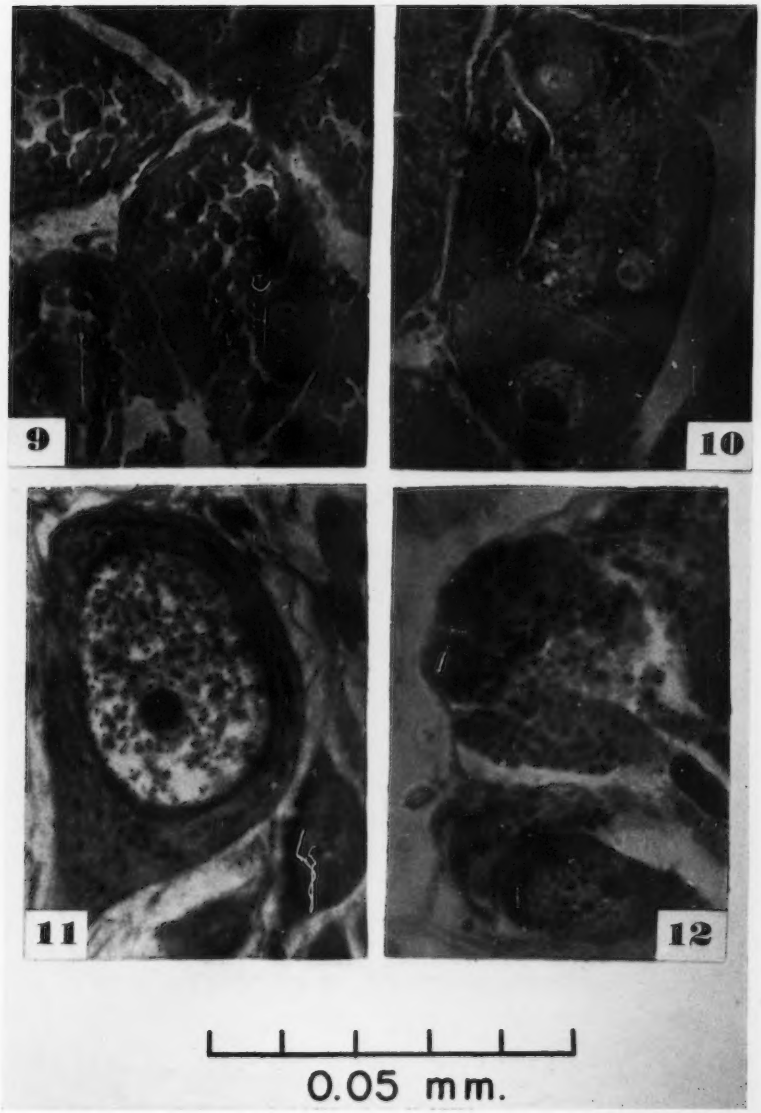

FIG. 9. Acinus of normal animal with a single egg and many clumps of spermatocytes and sperms. FIG. 10. Acinus of animal in which the tentacles were removed, showing four large eggs. FIG. 11. Neurone of suboesophageal ganglion showing secretory droplets in the cytoplasm of the cell and in the axon. Single nucleolus present. FIG. 12. Glandular cells of the collar of the optic, containing large droplets of neurosecretory material.

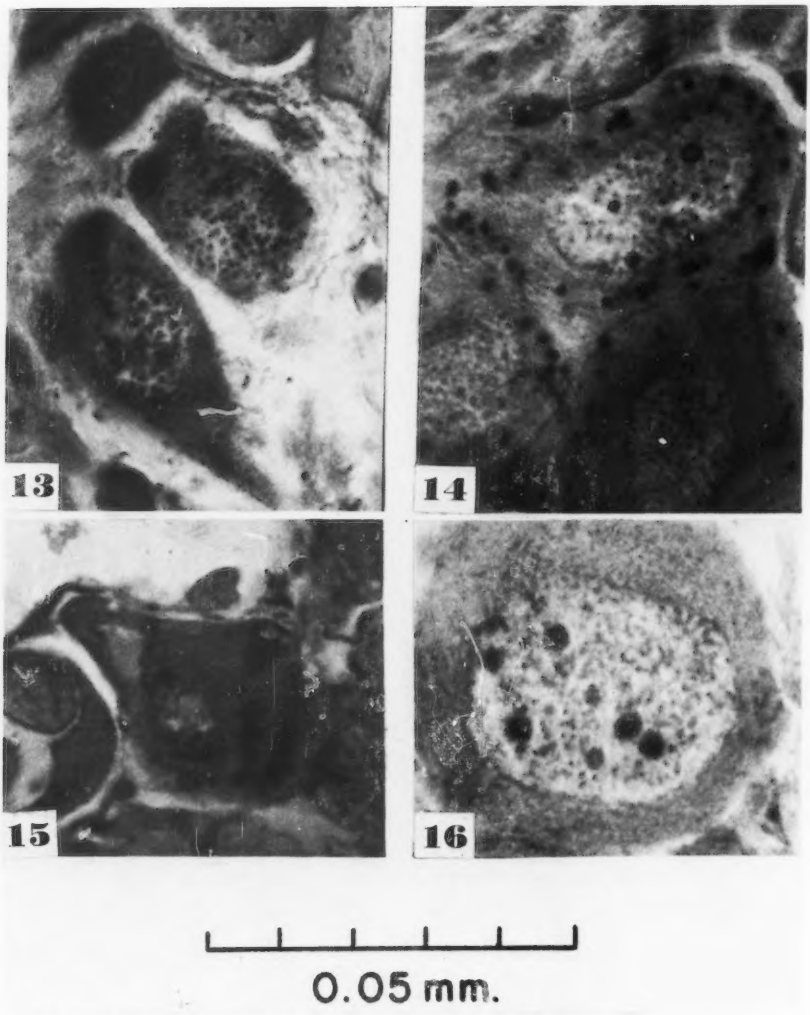

FIG. 13. Smaller neurone of the suboesophageal ganglion, showing secretory granules in the axon. FIG. 14. Golgi preparation, showing osmiophilic rods and platelets. FIG. 15. Glandular collar cell of the optic tentacle, showing large number of peripheral vacuoles from which neurosecretory material has been extruded. FIG. 16. Large neurone from the suboesophageal ganglion, showing many nucleoli and endopolyploid nucleus. The cytoplasm contains fine granular mitochondria.

cells forming this capsule have large nuclei with a small amount of cytoplasm. The nuclei of these cells react differently to staining, since one type of nucleus stains lightly with orange G and the other takes up light green. The two types of cell are arranged in short branching rows, according to their staining reactions.

The large and medium-sized cells have nuclei with a great variation in the number of nucleoli, which may range from 1 or 2 up to 20 or 30 in some of the largest cells. An example of a cell with nine visible nucleoli is well shown in Fig. 16. Staining with Pappenheim's methyl green pyronine, which is generally accepted as being specific for RNA and DNA, gave purple nucleoli (RNA) in a green nucleus (DNA). These nucleoli were Feulgen-negative, taking up only the light green counterstain. The variation in number of nucleoli is undoubtedly associated with the fact that these nerve cells show a high degree of endopolyploidy. Heitz (7) worked on 12 genera of pulmonate gasteropods including *Limax*, where he found that the giant nuclei of the ganglia contained many strongly condensed and individually recognizable chromosomes. In order to confirm this observation in the genus *Arion*, counts were made of the chromosome numbers in the nerve cells. The number of chromosomes counted with an ocular disk divided into squares ranged from 23 in the smallest cells to 1321 chromosomes in the largest nuclei. The occurrence of polysomaty in these nerve cells is suggestive perhaps of a relation between secretion and protein synthesis. Figure 16 shows many of these chromosomes.

Neurosecretory material occurs in neurones of all sizes, with the exceptions previously mentioned, in the form of droplets in the cytoplasm surrounding the nucleus and extending into the axon, which is clearly seen in Fig. 11 and again in Fig. 13, in a small neurone. These nerve cells showed the expected presence of mitochondria and Golgi substance, using that term in the "old-fashioned" sense. The appearance of the osmiophilic Golgi material in the form of spheres, rods, and platelets can be seen in Fig. 14, which also illustrates the variations in nuclear size and shape. Neurofibrillae appeared as faint striations within the cytoplasm, reaching out to the axonal strands. Nissl bodies appeared as ill-defined granular masses situated along the sides of the nucleus. The test for polysaccharides (PAS) showed faintly staining diffuse areas with no large individually recognizable granules. A summary of all the staining methods is given in Tables III and IV.

Live preparations confirmed the presence of nucleoli and mitochondria seen in fixed preparations, while methylene blue staining showed arcs, half-moons, and rings, whose central core stained with neutral red. It is not apparent without further experimental work what function, if any, these inclusions have in secretory activity.

B. The Optic Tentacles

Since cutting off the optic tentacles resulted in an increase in the number of eggs as well as in suppression of the growth of the ovotestis as a whole, it is of interest to look at the details of the structure of the tentacle and its nerve supply. Each tentacle consists of a sheath which encloses the larger tentacular nerve and the smaller optic nerve. At the tip of the tentacle there is a bulbous digitate ganglion which lies against the eye. The nerves are accompanied by

TABLE IV

Summary of cytoplasmic staining reactions in the nervous tissue

Type of cell	Cytoplasm	Secretory droplets	Mitochondria	Golgi
Fixed Cells				
Neurones of the brain	Diffusely granular and sometimes vacuolated	+ aldehyde fuchsin alone or in aggregations or in droplet form	Granules or small spheroids perinuclearly arranged or gathered at one end of the cell	Many rod-like, banana-shaped, or spherical forms arranged perinuclearly and throughout the entire cytoplasm in the larger cells
Cells of supraoesophageal ganglion	Very little around relatively larger nucleus	Scattered small droplets around nucleus in sparse cytoplasm	A few granules scattered throughout the cytoplasm around the nucleus	A few darkly staining spheres and rods in the scanty cytoplasm
Cells in fifth lobe of infraoesophageal ganglion	Fine granular appearance	Few or no aldehyde fuchsinophil granules, some granules which stain heavily with orange G	Small spheres and granules arranged around the nucleus	Many small spheres and rods scattered throughout the cytoplasm
Glandular collar cells of optic tentacle	Heavily granulated and sometimes vacuolated	Scattered aldehyde fuchsinophil granules, also larger spheroidal droplets + orange G + haematoxylin + acid fuchsin	Granules of varying sizes scattered diffusely around the nucleus	Dark-staining spheres and plates of varying size, arranged singly or clumped together, mainly aggregated in one end of the cell
Live Cells				
Neurones of the brain	Granular with many spheroids and minute vibrating particles	Stain with neutral red in center of larger duplex or mulberry spheroids	Small granules stained + with Janus green	Duplex spheres with a neutral red + medulla and a methylene blue + cortex; of varying sizes arranged throughout the cytoplasm around the nucleus
Glandular collar cells of optic tentacle	Full of spheroids which appear duplex	Neutral-red stain-center of clumps of spheroids	Minute granules in cloud-like formations at one end of cell	Neutral-red staining spheroids with an outer cortex and sometimes granules attached singly arranged or in groups

blood vessels and both these structures, before they reach the eye and its digitate ganglion, are surrounded by glandular cells, described in Table III. The cells are pear- or rectangular-shaped with a clear large nucleus, single nucleolus, and rather vacuolated and granular cytoplasm, shown in Fig. 15. These cells of the glandular collar are often crowded with secretory bodies which are acid-fuchsinophilic and stainable with orange G (Fig. 12). The vacuoles, which are often apparent in the cytoplasm, may represent areas from which the secretory material has been released. These vacuoles are visible in Fig. 15, at the periphery of the cell. There are also a few paraldehyde fuchsinophilic granules scattered throughout the cells, some of which have been seen in stained sections entering the blood vessels which run through this cellular area. The nuclei of these cells apparently give two different kinds of staining reaction; one type gives staining with orange G in light-green-staining cytoplasm, while the other stains with aldehyde-fuchsin in an orange-G-staining cytoplasm.

These glandular collar cells were examined weeks after the optic and tentacular nerves had been cut, by making a small incision in the dorsal surface of the body wall and snipping the two nerves. The skin was pushed together and the cut covered by the mantle fold. The optic nerve apparently regenerated after 3 or 4 weeks. Animals in which the optic and tentacular nerves had been cut by internal dissection were examined after several weeks and the gland cells showed some accumulation of secretion droplets. It is possible that the eye and its sensory ganglion receives information which is conveyed to the brain, which in turn controls the expulsion of secretory products from the glandular collar cells into the blood system.

In very young animals, secretory activity appeared to be very intense, the cells being very full of droplets, while in older animals there were fewer granules present, but many more vacuolated areas could be seen. It would be expected that at maturity there would be less intense secretion than in the growing animal.

The results of staining live preparations are summarized in Tables III and IV.

Discussion

The fact of the increase in the number of eggs following the cutting of the tentacles and their nervous tissue suggests that these produce a hormone which may suppress egg production while stimulating sperm production. Since these slugs are protandrous, such a mechanism would account for the development of sperm cells to the exclusion of eggs in young animals. It is also suggested that egg production is under the control of a hormone produced by the brain since the addition of brain homogenate also increased the egg production. One might draw up a theoretical picture on the basis of two hormones acting on the germinal epithelium, in the following way: It is assumed that both hormones are produced in the young animal, but in protandrous species the TH suppresses the EH and only sperms are formed until maturation sets in, when the EH now becomes active and is no longer inhibited by the TH. In the adult stage there is a balance between the two hormones, which differ from each other, since relatively few eggs in each follicle are produced, while

sperms are continually being formed. The cellular basis for these two hormonal systems is presumed to lie in the neurosecretory cells of the tentacle and the brain which have been described earlier in this paper.

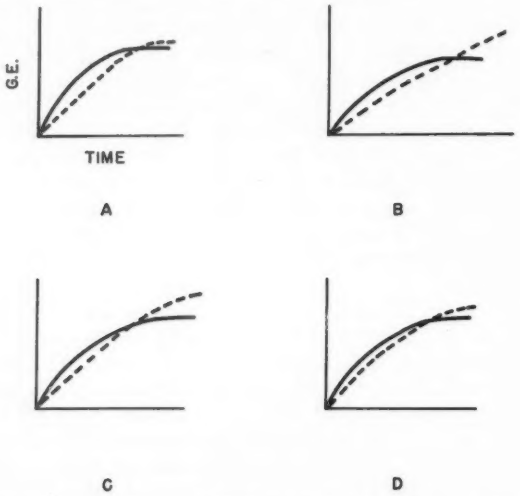

FIG. 17.———Tentacle hormone (TH). – – – – – – Egg hormone from brain (EH). G. E. = germinal epithelium. Time refers to developing gonad.

A. Normal animal. TH controls the production of the male germ cells from G. E. and also controls the production of EH until maturity.

B. Three to four weeks after cutting off the tentacles. The inhibitory effect of TH is removed and EH becomes active, resulting in many eggs being formed.

C. Three weeks after injecting brain tissue solution. Increased amount of EH overcomes inhibitory effect of TH and many eggs are produced.

D. Three weeks after injecting tentacle solution. Inhibitory effect of TH reduces normal growth rate of young animals. No increase in number of eggs.

Acknowledgments

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THE MONONCHIDAE: A FAMILY OF PREDACEOUS NEMATODES

II. GENUS ANATONCHUS (ENOPLIDA: MONONCHIDAE)[1]

ROLAND H. MULVEY

Abstract

Anatonchus ginglymodontus n. sp., *A. subacutus* n. sp., *A. alleni* n. sp., and *Anatonchus* sp., near *alleni*, are described and figured. The female of *Anatonchus gracilicaudatus* (Cobb, 1917) is described and figured for the first time. The development of the head and tail structures in juvenile stages of *Anatonchus tridentatus* (de Man, 1876) and *A. dolichurus* (Ditlevsen, 1911) is described and figured. A key to the species of the genus *Anatonchus* is provided.

Introduction

This paper is the second in a series dealing with the taxonomy of the Mononchidae, a family of free-living predatory nematodes inhabiting soil and fresh water. The genus *Anatonchus* (Cobb, 1916) de Coninck, 1939 was first established by Cobb as a subgenus. Cobb (2) included three species in his monograph, viz., *Mononchus* (*Anatonchus*) *tridentatus* de Man, 1876, *M.* (*A.*) *dolichurus* Ditlevsen, 1911, and *M.* (*A.*) *gracilicaudatus*, a new species. de Coninck (3) in describing and illustrating a male and several juveniles of *A. tridentatus* was the first to regard *Anatonchus* at the generic level. Andrássy (1) proposed the new combinations of *A. tridentatus*, *A. dolichurus*, and *A. gracilicaudatus*. However, he apparently did not consider de Coninck's paper of 1939 although he included it in his list of references. The validity of *A. gracilicaudatus* has been questioned by several workers including Micoletzky (12) and Ditlevsen (5). Hofmänner and Menzel (7) and Micoletzky (12) differed in their opinions as to the number of teeth in the buccal cavity of *A. dolichurus*. The present paper attempts to deal with these differences and also with establishing some new species in this genus.

Materials and Methods

Collection, preparation of slide mounts, measurement, and illustration of nematodes were done as previously described by Mulvey (13).

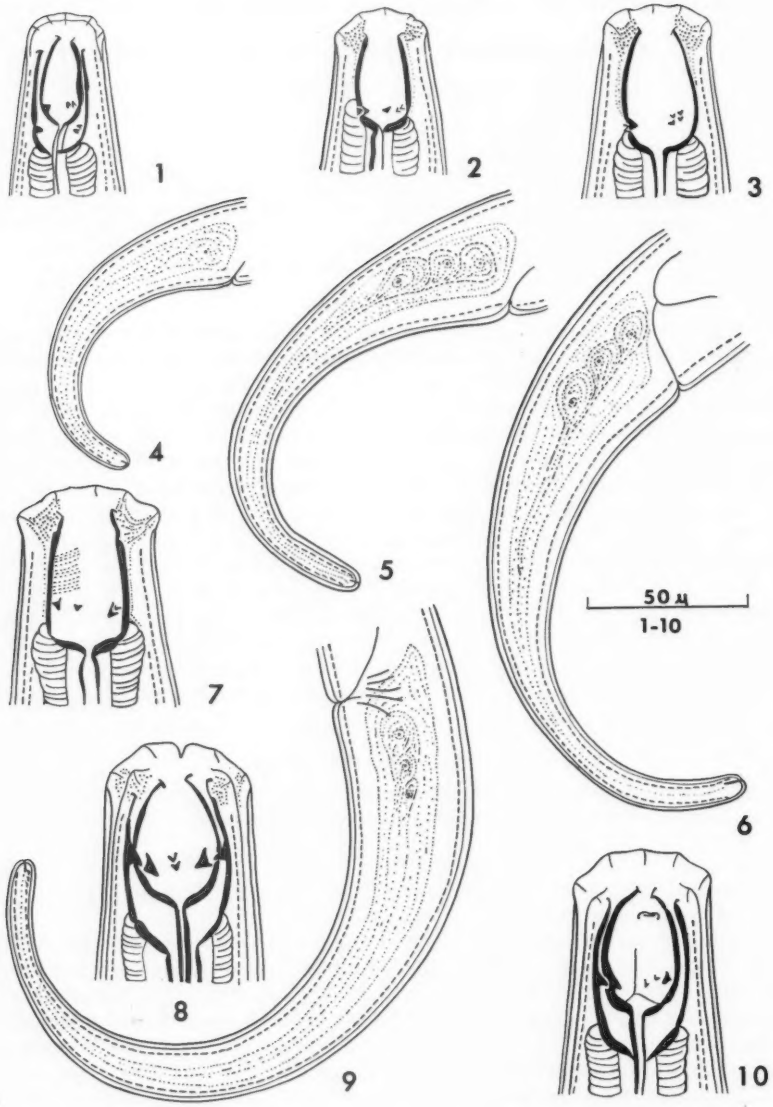
Anatomical Studies

Position and Direction of the Teeth in A. tridentatus and A. dolichurus

(*a*) *A. tridentatus*

de Man (9) first described and figured males and females of this species. His original illustrations show three equal, fairly strong retrorse teeth located in the anterior third of the buccal cavity. In 1921 de Man (10) described and illustrated the head only of a female of this species. He also figured the head of a juvenile (probably the third stage) in which the three retrorse teeth were situated in the posterior third of the buccal cavity. His 1921 illustration of the female shows a large amphid located just anterior to the three teeth. Cobb (2)

[1]Manuscript received June 12, 1961.
Contribution from the Nematology Section, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.



FIGS. 1–10. *Anatonchus tridentatus*, juvenile stages (Figs. 1–7, 9, specimens from Holland). 1, 4. First stage, molting. 2, 5. Second stage. 3, 6. Third stage. 7, 9. Fourth stage. 8. Fourth stage, molting (female from France). 10. Fourth stage, molting (male from England).

TABLE I

Measurements of juveniles and adults of *Anatonchus dolichurus* from Canada and *A. tridentatus* from Holland

	Species	No. of specimens	Body length (mm)	Tail length (mm)	*c*-value	Buccal cavity	
						Length (μ)	Width (μ)
Juvenile stage							
1	*dolichurus*	3	1.2–1.8	0.29–0.36	4.3–5.0	35–40	18–28
	tridentatus	1	0.83	0.12	6.9	27	11
2	*dolichurus*	4	2.1–2.6	0.46–0.58	4.2–5.2	53–55	29–38
	tridentatus	1	1.4	0.16	8.7	32	17
3	*dolichurus*	2	3.7–3.8	0.70–0.75	5.0–5.4	68–70	42–43
	tridentatus	1	1.6	0.19	7.4	39	23
4	*dolichurus*	1	4.5	0.98	4.6	83	50
	tridentatus	2	2.0	0.18–0.19	10.5–11.0	40	20–22
Adult (females)	*dolichurus*	1	6.3	1.4	4.5	100	60
	tridentatus	5	1.8–2.7	0.18–0.29	8.0–10.8	42–60	32–34

used de Man's illustration of 1876 of the female in his description of this species but apparently Cobb had not found *A. tridentatus* in soil in the United States. Goodey (6) illustrated and described in detail males and females of this species found in England. He emphasized the presence of three sharp retrorse teeth situated in the anterior third of the buccal cavity. Kreis (8) described and illustrated a female which deviated considerably in the details of the buccal cavity from that of de Man (9) and therefore this specimen of Kreis is best regarded as *incertae sedis*. Hofmänner and Menzel (7) and Stefanski (14) noted that the three retrorse teeth in the adult were situated somewhat anterior to the center of the buccal cavity.

I examined 18 males and 35 females from various European countries and observed that the three retrorse teeth in the males were about midway in the buccal cavity and in the females they were in the anterior third of the buccal cavity.

Preserved specimens from Holland were used in the study of the development of the buccal cavity and tail (Figs. 1–7, 9) from juvenile to adult. Specimens from France, England, and Germany were studied for comparison. The studies showed that the teeth of *A. tridentatus* are directed anteriorly in the first-stage juvenile but become retrorse during the first molt. In the remaining stages the teeth are retrorse. However, the three teeth do not assume their normal position until the last molt (Figs. 8, 10). Tail growth during the juvenile stages (Table I) is much less in proportion to that of *A. dolichurus*.

(*b*) *A. dolichurus*

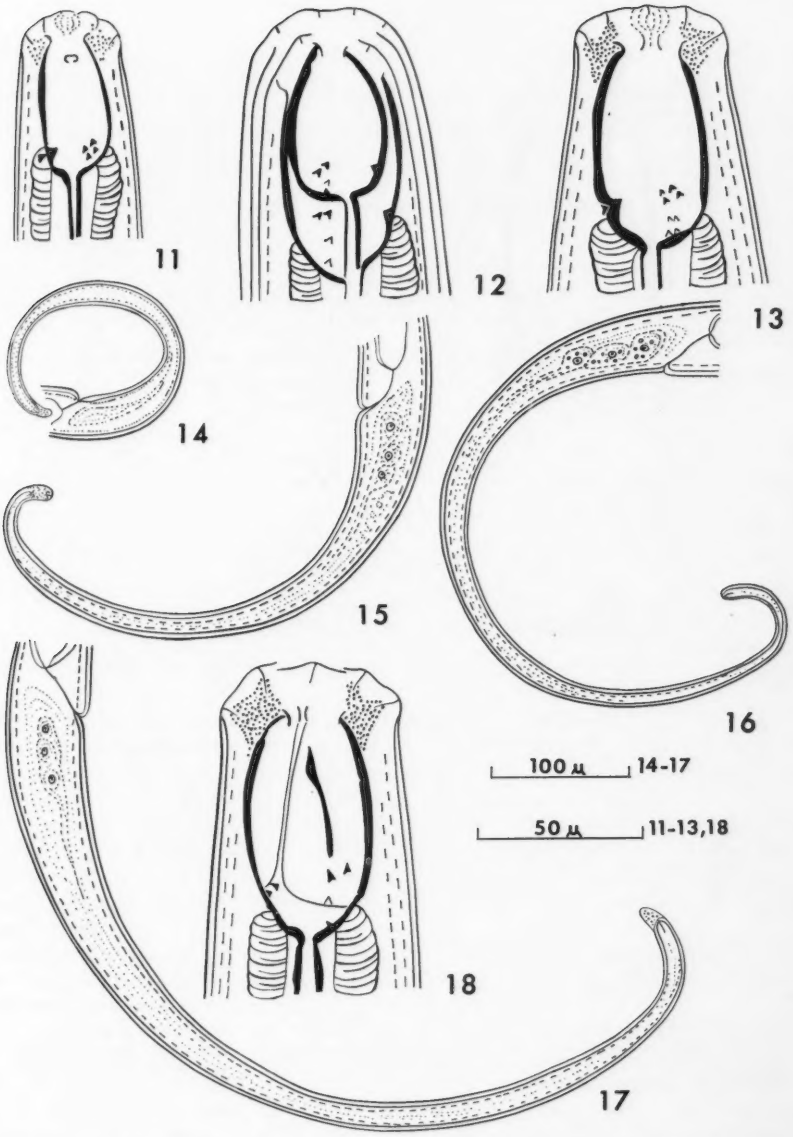
Ditlevsen (4) first described and illustrated this species. His illustration showed a fourth-stage molting juvenile. The two sets of posterior-pointed structures are probably angular foramina similar to that described by Goodey (6) in the buccal cavity of the adult *A. tridentatus*. Hofmänner and Menzel (7) surmised that the specimen examined by Ditlevsen (4) had been molting. Their sexually mature female specimens of *A. dolichurus* had only three teeth. Menzel (11) was the first to illustrate and describe adult females of this species. His Swiss specimens had three equally large retrorse teeth situated in the posterior third section of the buccal cavity. Micoletzky (12) disagreed with Hofmänner and Menzel's (7) presumption that Ditlevsen's specimen of 1911 represented a molting stage. Juveniles for the study of the development of the head and tail (Figs. 11–18, 19–22) of *A. dolichurus* were obtained from a population taken from bog soil near Ottawa, Canada. These were subsequently killed, fixed, and mounted on slides in glycerin. In *A. dolichurus* the three teeth of the juvenile stages point forward until the final molt. The tail of this species undergoes considerable growth in length during the development of the juvenile to the adult stage (Table I). The results of this study confirm Hofmänner and Menzel's (7) statements.

Taxonomy

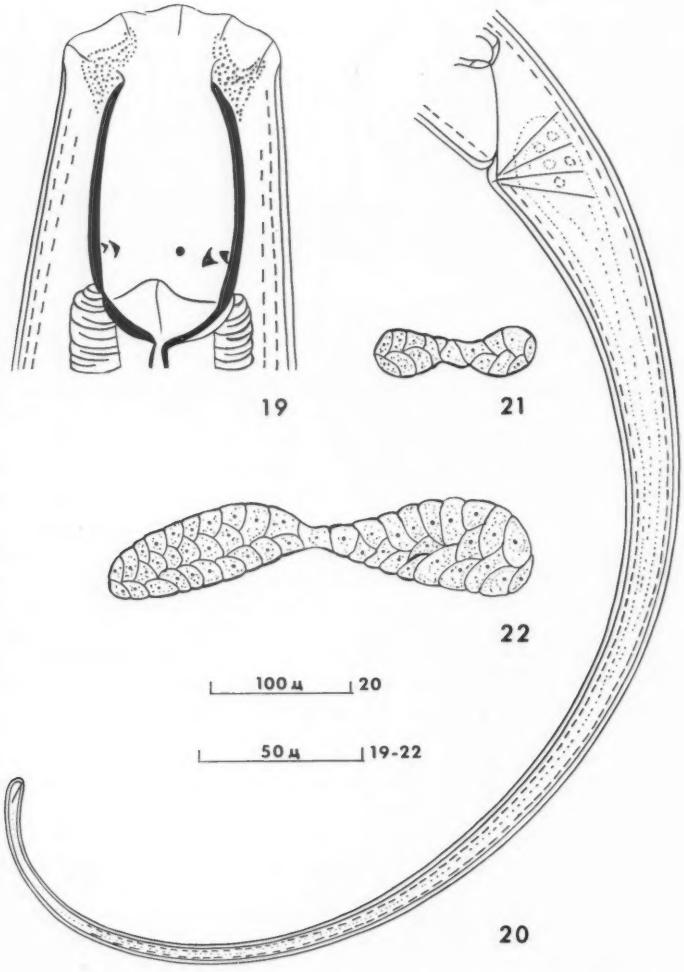
Genus *Anatonchus* (Cobb, 1916) de Coninck, 1939

Diagnosis.—Teeth retrorse, situated anteriorly to nearly basal in the buccal cavity, generally large species with roomy buccal cavity, tail long and usually becoming cylindroid, female organs paired, males of most species known.

Type species.—*Anatonchus tridentatus* (de Man, 1876) de Coninck, 1939.



FIGS. 11–18. *Anatonchus dolichurus*, juvenile stages (all specimens from Ontario, Canada). 11, 14. First stage. 12, 15. First stage, molting. 13, 16. Second stage. 17, 18. Third stage.



FIGS. 19–22. *Anatonchus dolichurus*, fourth-stage juvenile (specimen from Ontario, Canada). 19. Head. 20. Tail. 21. Developing ovaries of second-stage juvenile. 22. Developing ovaries of fourth-stage juvenile.

KEY TO SPECIES OF GENUS *Anatonchus*

FEMALES

1. Teeth midway to anterior in buccal cavity 2
   Teeth nearly basal in buccal cavity 4
2. Species very long and thin (L = 5.3 mm; a = 71.0), buccal cavity large (75×65 μ) ..... *gracilicaudatus* (Cobb)
   Species shorter and wider (body length generally less than 3 mm), buccal cavity medium-sized 3
3. Teeth nearly anterior, appear hinged to walls of buccal cavity, buccal cavity nearly as wide as long ..... *ginglymodontus* n. sp.
   Teeth situated in anterior third of buccal cavity, not hinged, buccal cavity considerably longer than wide ..... *tridentatus* (de Man)
4. Tail very long (c = 4.5–5.5) ..... *dolichurus* (Ditlevsen)
   Tail relatively short (c = 6.7–11.3) 5
5. Tail terminus acutely rounded, spinneret absent ..... *subacutus* n. sp.
   Tail terminus rounded, terminal spinneret present 4
6. Body length 2.5–3.5 mm, buccal cavity 50–58 μ long × 35–50 μ wide, with thin walls ..... *alleni* n. sp.
   Body length 4.0–4.8 mm, buccal cavity 75–80 μ long × 52–58 μ wide, walls thick ..... *Anatonchus* sp. near *alleni*

MALES

1. Teeth midway in buccal cavity 2
   Teeth nearly basal in buccal cavity 3
2. Species long, thin (L = 6.1 mm; a = 56.0), buccal cavity 93×72 μ, spicule length about 140 μ ..... *gracilicaudatus* (Cobb)
   Species shorter and wider (L = 1.7–2.6 mm, a = 33 or less), buccal cavity 40–55 μ long × 22–35 μ wide, spicule length 83–110 μ ..... *tridentatus* (de Man)
3. Tail very long (c = 5.5), spicule length about 160 μ ..... *dolichurus* (Ditlevsen)
   Tail shorter (c = 8.1–10.4), spicule length 84–125 μ 4
4. Tail terminus acute, spinneret absent ..... *subacutus* n. sp.
   Tail terminus rounded, terminal spinneret present 5
5. Length 2.7–3.0 mm, buccal cavity 49–52 μ long × 37–40 μ wide, spicule length 90–105 μ ..... *alleni* n. sp.
   Length 3.8–4.6 mm, buccal cavity 70–75 μ long × 50 μ wide, spicule length 125–130 μ ..... *Anatonchus* sp., near *alleni*

Anatonchus tridentatus (de Man, 1876) de Coninck, 1939 (Figs. 23–25)

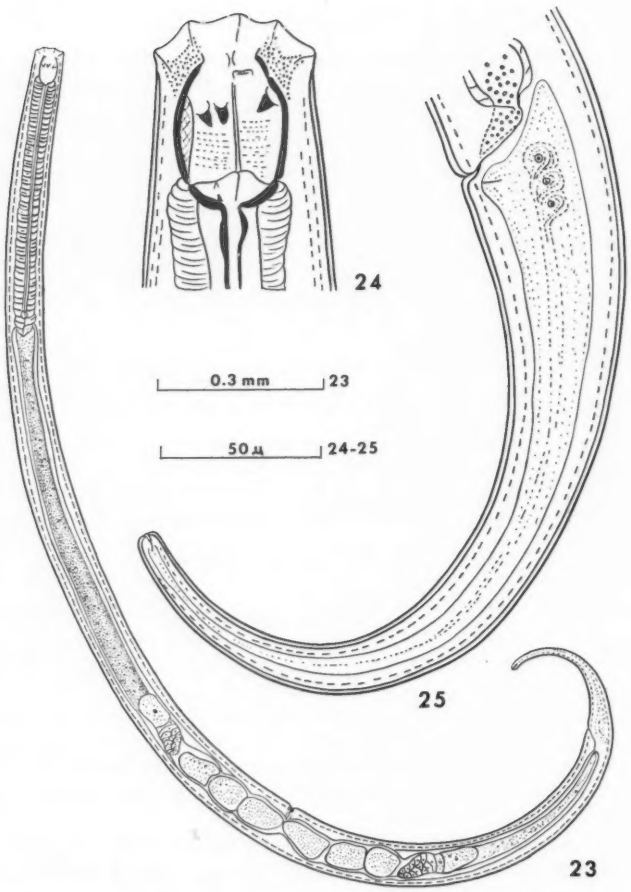
California specimens (2 females).—L = 2.3–2.6 mm; a = 31–32.5; b = 4.8–5.2; c = 7.7–8.6; V = 61%; buccal cavity = 48–49 × 34–36 μ; tail length = 0.30 mm.

These specimens from California generally conform to de Man's description (9). Only two females and two juveniles were available for study. Sperm was not observed in the female reproductive tracts. Caudal glands are obscure in the adults but distinct in the juveniles. The teeth of the juveniles are basal and retrorse.

Males and females of *A. tridentatus* from Holland, Switzerland, Germany, France, and England were studied in detail. Measurements of these specimens are contained in Table II.

A. tridentatus has a rather wide range in body length and buccal cavity size (Table II). de Man (9) records females slightly greater than 3 mm in body length. Hofmänner and Menzel (7) stated that the females they examined from Switzerland ranged from 1.55 to 2.33 mm. I examined a male and a female from Switzerland and found that the female body length was over 3 mm.

Habitat.—Turf, forest and orchard soil, flower garden soil.

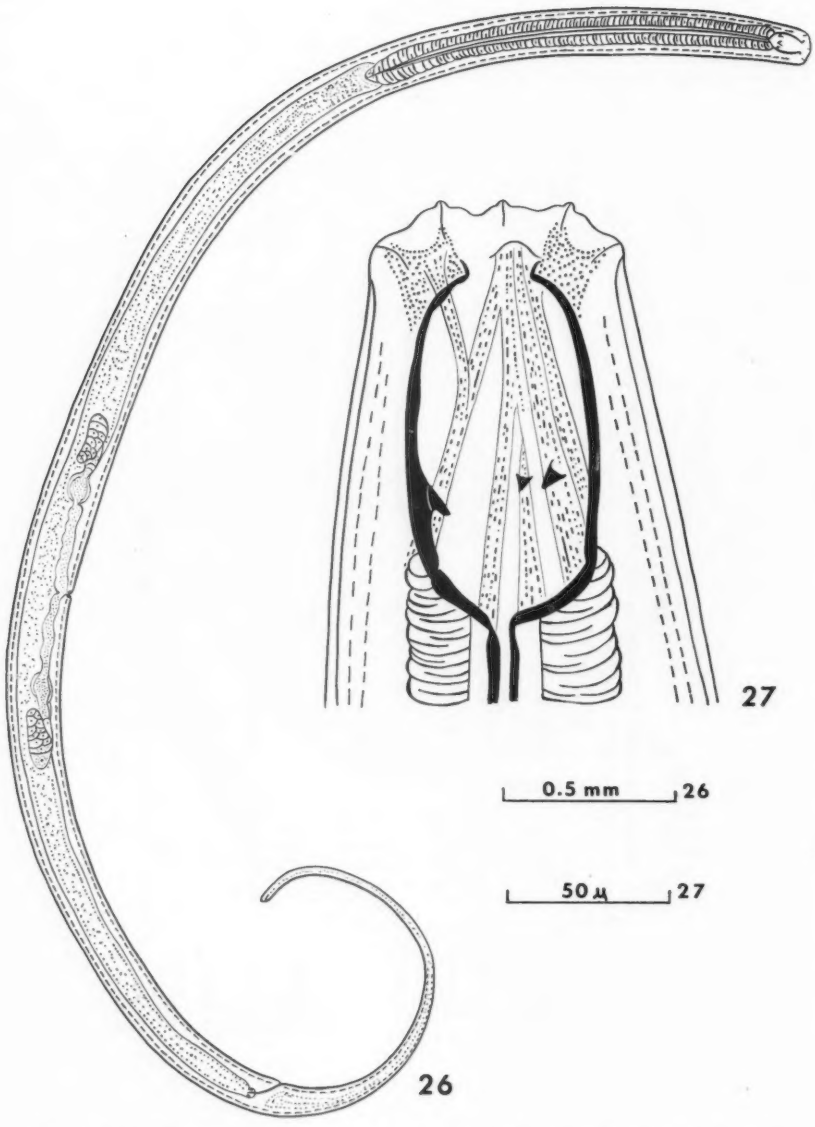


FIGS. 23–25. *Anatonchus tridentatus* (specimen from California, U.S.A.). 23. Female. 24, 25. Female head and tail enlarged.

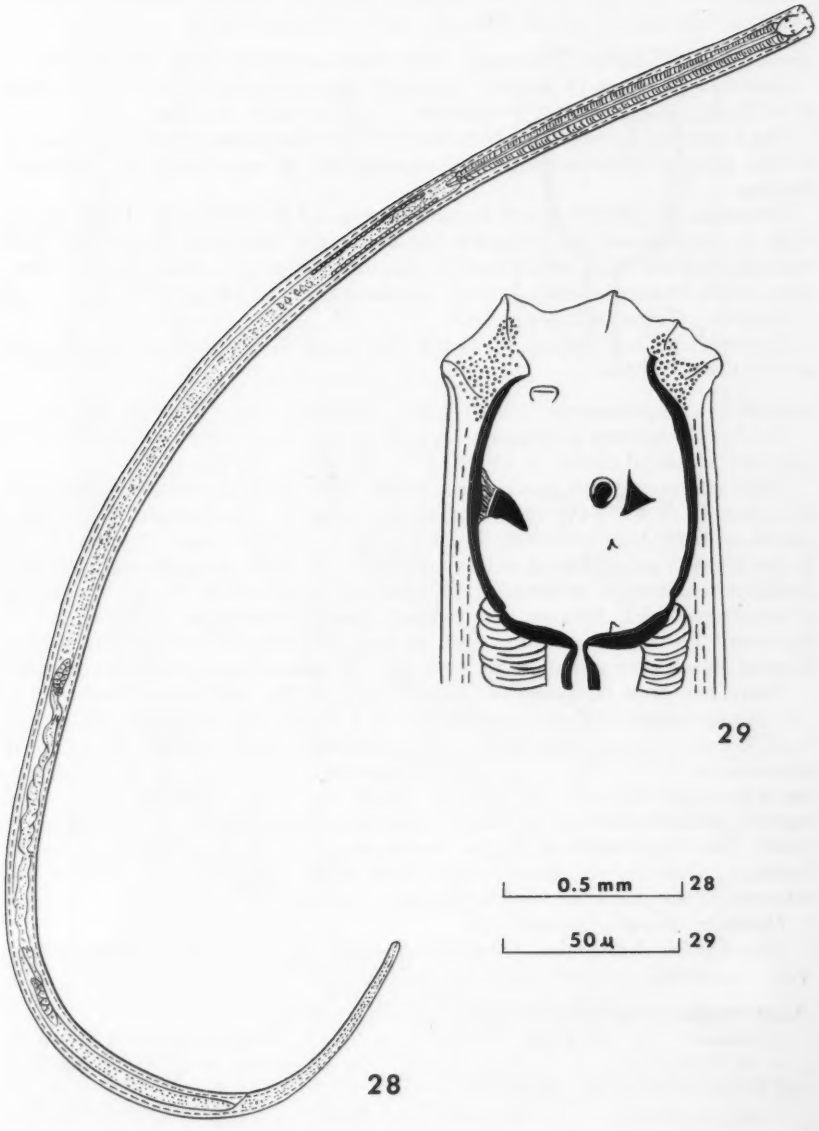
TABLE II

Measurements of *A. tridentatus* from various European countries

Country	No. of specimens	Body length (mm)	Buccal cavity		Tail length (mm)	*c*-value	Spicule length (μ)	*V* (%)
			Length (μ)	Width (μ)				
Holland	5 females	2.3 (1.8–2.7)	42–60	32–34	0.24 (0.18–0.29)	9.6 (8.0–10.8)	—	61 (60–62)
	7 males	2.1 (1.7–2.4)	40–50	22–35	0.19 (0.13–0.25)	11.6 (9.7–13.8)	85–100	—
England	5 females	2.3 (2.2–2.5)	44–52	31–42	0.25 (0.19–0.27)	9.5 (7.0–11.8)	—	61 (59–62)
	3 males	2.2 (2.2–2.3)	40–44	25–30	0.18 (0.15–0.20)	12.5 (11.2–14.3)	80–100	—
Germany	1 female	2.5	48	38	0.27	9.3	—	61
	1 male	2.3	45	31	0.19	11.5	83	—
France	7 females	2.4 (2.1–2.5)	42–50	30–33	0.24 (0.20–0.27)	9.7 (9.3–10.5)	—	63 (60–64)
	1 male	2.4	40	28	0.19	12.6	95	—
Switzerland	1 female	3.4	60	42	0.43	7.9	—	64
	1 male	2.6	55	35	0.32	8.1	110	—



FIGS. 26 and 27. *Anatonchus dolichurus* (specimen from Ontario, Canada). 26. Female. 27. Female head enlarged.



FIGS. 28 and 29. *Anatonchus gracilicaudatus* (specimen from California, U.S.A.). 28. Female. 29. Female head enlarged.

Geographical distribution.—Switzerland, Germany, Austria, Holland, France, Belgium, England, Scotland, Mexico, and California, U.S.A.

Anatonchus dolichurus (Ditlevsen, 1911) Andrássy, 1958 (Figs. 26 and 27)

Canadian specimen (1 female).—L = 6.3 mm; a = 42.0; b = 4.7; c = 4.5; V = 51.6%; buccal cavity = 100×60 μ; tail length = 1.4 mm.

The Canadian specimen fits Ditlevsen's 1928 description (5) of adult females of this species. Ditlevsen's original description (4) was based on immature females.

Ditlevsen (5) was the first to record the male of *A. dolichurus*. He found one male in a population of 25 females. He adequately described and figured (tail and spicules) the male, which was 6.3 mm long and had a c value of 5.5. There were about 20 supplements and the spicule was about 160 μ in length.

Habitat.—Green turf, boggy soil.

Geographical distribution.—Jutland, Switzerland, Norway, Faroes Island, and Ottawa, Canada.

Anatonchus gracilicaudatus (Cobb, 1917) Andrássy, 1958 (Figs. 28 and 29)

California specimen (1 female).—L = 5.30 mm; a = 70.6; b = 4.8; c = 7.9; V = 65%; buccal cavity = 75×65 μ; tail length = 0.67 mm.

Cobb (2) erected this species on a single male. The following description is of a female that I have identified as belonging to this species: Labia conspicuous; body long and thin. Three strong, equal-sized teeth about midway in the buccal cavity. Buccal cavity spacious, with walls of moderate thickness. Longitudinal muscle structure prominent as described by Cobb (2). Lining of esophagus thick. Ovaries two, reflexed about one quarter, prominent valve between uterus and oviduct. Tail arcuate, tapering to rounded terminus. Caudal glands three, small, in tandem. Spinneret opening terminal but obscure.

There has been considerable discussion as to the validity of this species. Micoletzky (12) considered it a variety of *A. dolichurus*. Ditlevsen (5) stated that *A. gracilicaudatus* was probably identical with *A. dolichurus*. After careful examination of the illustrations of both species in the literature and the specimens available for study, I have concluded that *A. gracilicaudatus* is a valid species. It differs from *A. dolichurus* in having a larger buccal cavity, and larger teeth. The three teeth of *A. gracilicaudatus* are robust and situated nearly midway in the buccal cavity, while those of *A. dolichurus* are smaller and situated in the posterior third of the buccal cavity.

Habitat.—Marshy ground.

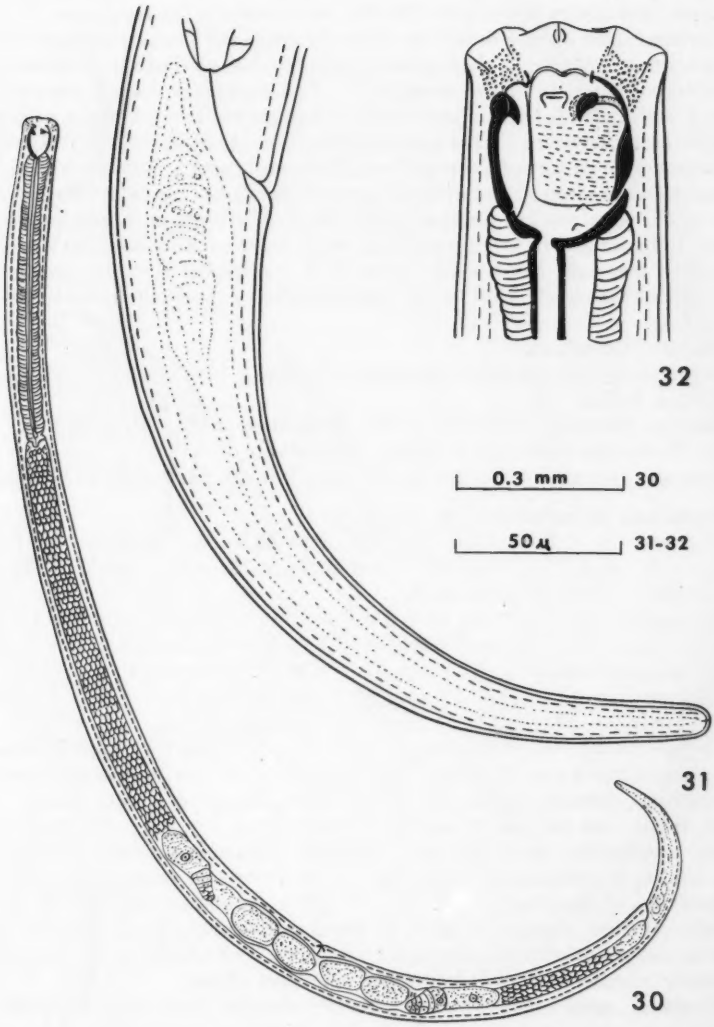
Geographical distribution.—Arlington Farm, Virginia, U.S.A. (male); Placerville, California (female).

Anatonchus ginglymodontus n. sp. (Figs. 30–32)

(4 females).—L = 2.4 mm (2.2–2.6); a = 30.7 (29.6–31.1); b = 4.4 (4.2–4.7); c = 10.3 (9.3–11.0); V = 63% (61–66); buccal cavity = 42–48 × 40–42 μ; tail length = 0.24 mm (0.22–0.26).

Holotype (female).—L = 2.6 mm; a = 30.6; b = 4.7; c = 10.4; V = 61%; buccal cavity = 48×42 μ; tail length = 0.25 mm.

Diagnosis.—Labia distinct, buccal cavity barrel-shaped, nearly as wide as long. Three medium-sized retrorse teeth, pointed, and appear to be hinged to



FIGS. 30–32. *Anatonchus ginglymodontus* n. sp. 30. Female. 31. Female tail enlarged. 33. Female head enlarged.

anterior wall of the buccal cavity. Wall of buccal cavity anterior to base of teeth weakly developed. Walls of buccal cavity distinctly striated, of medium thickness, and cavity leads into a thickly lined lumen of the esophagus. Ovaries two, reflexed, and having a distinct valve between the uterus and the oviduct. One specimen contained a thick-shelled egg ($100 \times 60\ \mu$). No sperm observed in reproductive tract. Intestine tessellated. Tail uniformly conoid, curved ventrally. Caudal glands leading into terminal spinneret. Tail terminus rounded.

Differential diagnosis.—This species differs from *A. tridentatus* in the position and attachment of teeth to the walls of the buccal cavity. The teeth in *A. ginglymodontus* are close to the anterior portion of the buccal cavity walls, while those of *A. tridentatus* are situated farther back. The teeth of *A. ginglymodontus* appear to be hinged to the buccal wall, while those of *A. tridentatus* appear to be part of the wall. The buccal cavity of *A. tridentatus* is rather longer than wide ($48 \times 38\ \mu$) while that of *A. ginglymodontus* is about as wide as long ($44 \times 42\ \mu$).

Habitat.—Grass sod.

Geographical distribution.—Berkeley Campus, University of California, California, U.S.A.

Holotype (*female*).—Collection 307. Deposited with University of California. Nematode collection at Davis, California.

Paratypes (*females*).—Collection 257, slide No. 20. Same data as holotype.

Anatonchus subacutus n. sp. (Figs. 33–37)

(*10 females*).—L = 2.6 mm (2.5–2.9); a = 40.9 (35.7–46.0); b = 4.7 (4.4–5.0); c = 8.7 (7.6–9.3); V = 64% (60–66); buccal cavity = 48–52 × 36–40 μ; tail length = 0.30 mm (0.28–0.34).

(*10 males*).—L = 2.5 mm (2.1–3.0); a = 41.9 (33.3–50.0); b = 5.0 (4.2–5.6); c = 9.8 (8.1–10.8); spicule length = 84 μ (80–90); buccal cavity = 42–50 × 31–38 μ; tail length = 0.25 mm (0.23–0.29); supplements = 12–13.

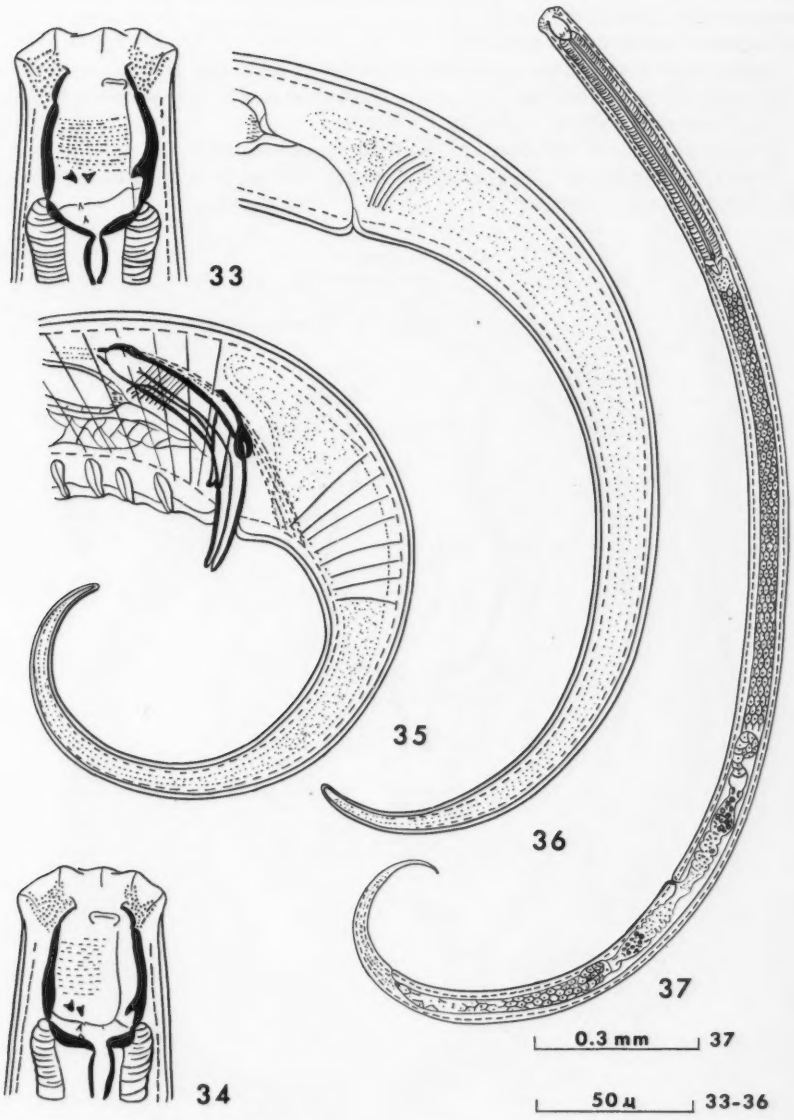
Holotype (*female*).—L = 2.5 mm; a = 35.7; b = 4.6; c = 8.3; V = 66%; buccal cavity = $50 \times 38\ \mu$; tail length = 0.30 mm.

Allotype (*male*).—L = 2.4 mm; a = 42.1; b = 5.0; c = 10.4; spicule length = 87 μ; buccal cavity = $42 \times 32\ \mu$; tail length = 0.23 mm; supplements = 13.

Diagnosis, female.—Labia set off by slight depression, teeth three, equal-sized, small, and located in posterior third of buccal cavity. Walls of buccal cavity moderately thick, distinctly striated. Amphids distinct, aperture slit-like. Lining of esophageal lumen thick, flared outward immediately posterior to junction of esophagus and buccal cavity. Intestine tessellated. Ovaries double, reflexed, spermathecae of all the females amply supplied with sperm. Uterus and spermatheca connected by a valve. Tail conoid, arcuate, terminus minutely rounded. Caudal glands and spinneret absent.

Diagnosis, male.—Buccal cavity usually smaller than that of the female. Testes two, outstretched, spicules bulky, accessory pieces bifurcated, gubernaculum bifid. Supplements elevated above level of cuticle. Tail sharply arcuate conoid ending in minutely rounded terminus. Caudal glands and spinneret absent.

Differential diagnosis.—*A. subacutus* differs from *A. tridentatus* in the position of teeth and details of tail structure and shape. The minutely rounded



FIGS. 33–37. *Anatonchus subacutus* n. sp. 33. Female head enlarged. 34. Male head enlarged. 35. Male tail enlarged. 36. Female tail enlarged. 37. Female.

tail terminus and absence of spinneret and caudal glands separates this species from all other species in the genus.

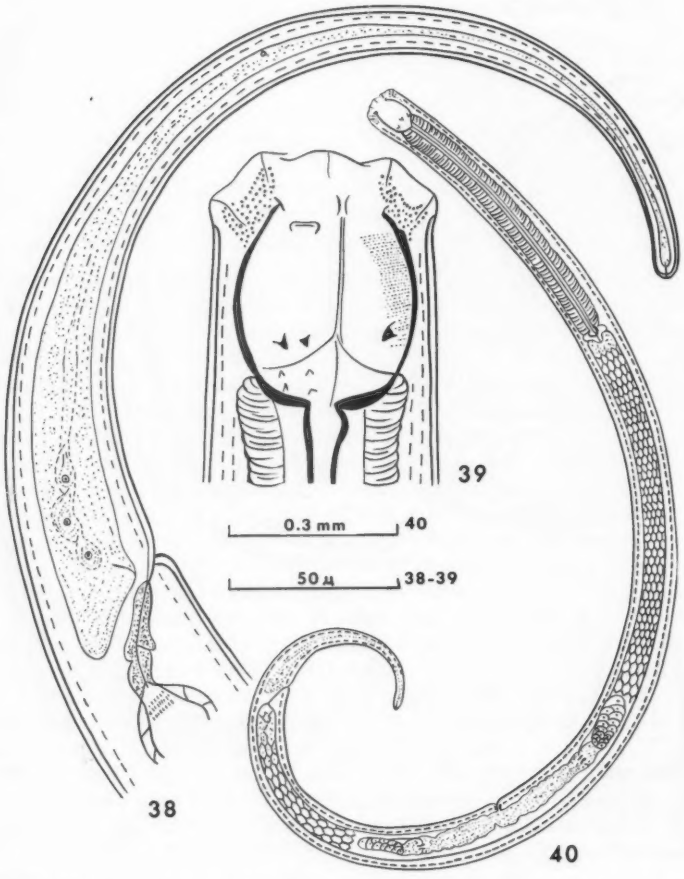
Habitat.—Soil beneath oak tree.

Geographical distribution.—Cordelia Junction, California, U.S.A.

Holotype (*female*).—Collection 162. Deposited with University of California Nematode collection, Davis, California.

Allotype (*male*).—Collection 162. Same data as holotype.

Paratypes (*males and females*).—Collection 162, slide Nos. 10*a*, 10*b*, and 10*c*. Same data as holotype.

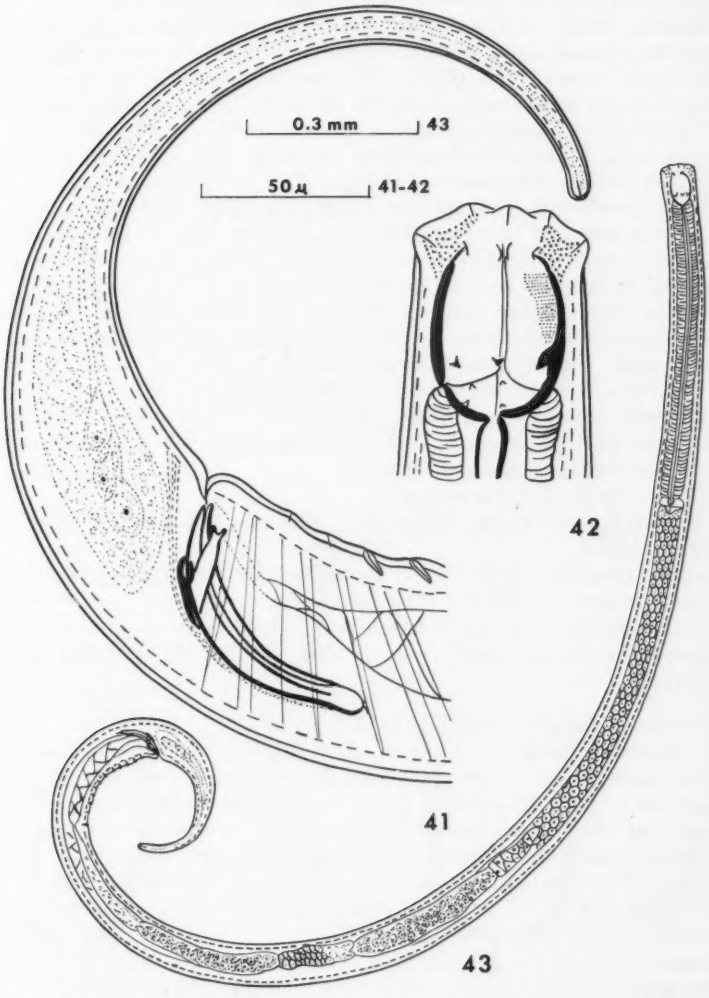


FIGS. 38–40. *Anatonchus alleni* n. sp. 38. Female tail enlarged. 39. Female head enlarged. 40. Female.

Anatonchus alleni[2] n. sp. (Figs. 38–40, 41–43)

(*7 females*).—L = 3.0 mm (2.5–3.5); a = 38.6 (33.3–50.0); b = 4.9 (4.4–5.5); c = 8.6 (6.7–11.3); V = 63% (60–66); buccal cavity = 50–58 × 35–50 μ; tail length = 0.35 mm (0.31–0.39).

(*3 males*).—L = 2.8 mm (2.7–3); a = 41.2 (37.5–47.6); b = 4.9 (4.7–5.5);



FIGS. 41–43. *Anatonchus alleni* n. sp. 41. Male tail enlarged. 42. Male head enlarged. 43. Male.

[2]In honor of Dr. M. W. Allen, University of California.

c = 9.4 (8.5–10.0); spicule length = 97 μ (90–105); buccal cavity = 49–52 × 37–40 μ; supplements = 12–17.

Holotype (*female*).—L = 2.5 mm; a = 34.0; b = 4.5; c = 7.1; V = 62%; buccal cavity = 58×50 μ; tail length = 0.35 mm.

Allotype (*male*).—L = 2.8 mm; a = 38.4; b = 4.7; c = 8.5; spicule length = 105 μ; buccal cavity = 50×40 μ; supplements = 13.

Diagnosis, female.—Labia distinct, buccal cavity roomy, longer than wide. Teeth three, retrorse, equal-sized, relatively small, and situated in the posterior third of buccal cavity. Walls of buccal cavity thin, obscurely striated. Junction of buccal cavity and esophagus flared outward, lining of esophageal lumen thick. Ovaries two, reflexed and with distinct valve between oviduct and uterus. Spermatheca in fertilized females large and filled with sperm. A thin-shelled egg in one female measured 120×70 μ. Tail uniformly conoid, ventrally arcuate, and ending in a rounded terminus. Caudal glands three, saccate, and leading to a terminal unarmed spinneret. Glands obscure in older females.

Diagnosis, male.—Details of head area same as those of female. Testes two, containing many small sperm. Three to four pairs of ejaculatory glands just anterior to the spicules. Spicules arcuate, relatively slender. Supplements slightly elevated above level of cuticle. Accessory pieces bifurcated, gubernaculum bifid. Caudal glands three, saccate, leading to terminal unarmed spinneret. Tail conoid arcuate with rounded terminus.

Differential diagnosis.—A. alleni differs from *A. tridentatus* in the position of teeth and relative proportions of buccal cavity. It may be separated from *A. subacutus* by tail shape and terminus.

Habitat.—Orchard and lawn soil.

Geographical distribution.—Berkeley, California, and Santa Clara County, California, U.S.A.

Holotype (*female*).—Coll. 303A. Deposited with University of California Nematode collection, Davis, California.

Allotype (*male*).—Coll. 303A, same data as holotype.

Paratypes (*males and females*).—Coll. 304, 188, 99, 257, and 165, same data as holotype.

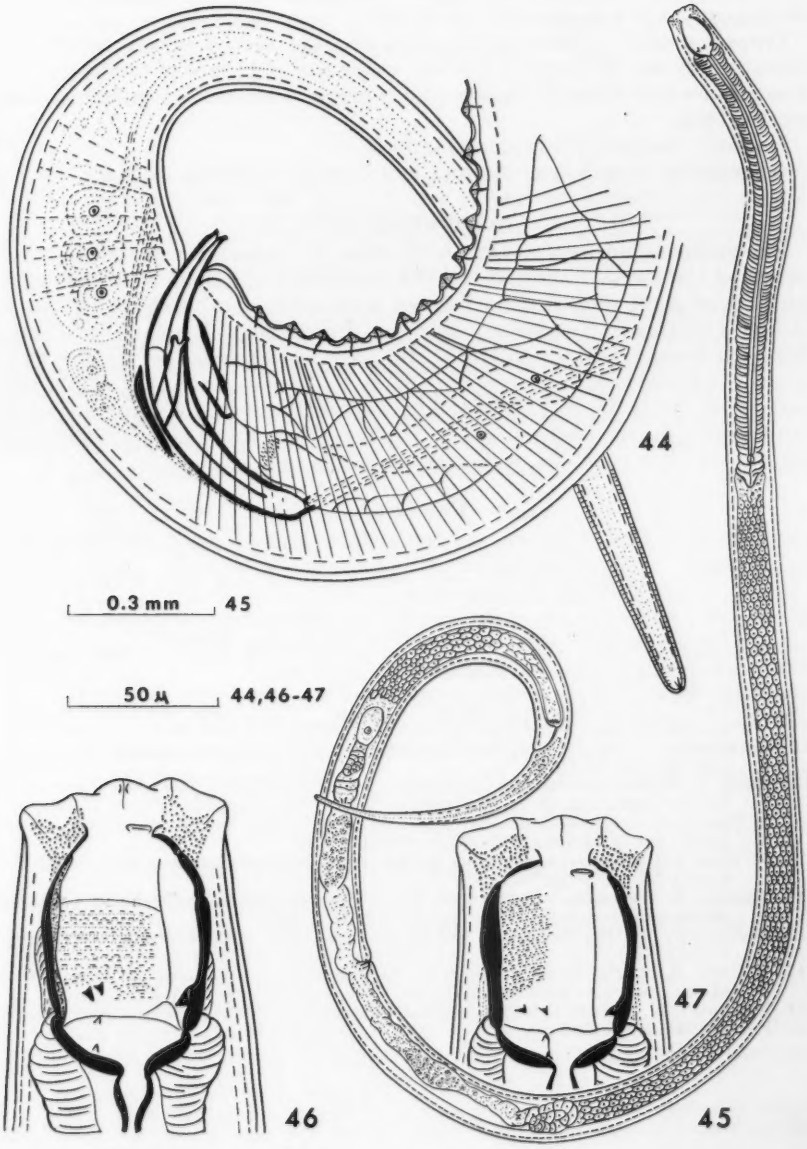
Anatonchus sp., near *alleni* (Figs. 44–47)

In addition to the specimens of *A. alleni* described there were three females and two males found in the same area which differed considerably from the new species in body length and buccal cavity size. At the present time I do not feel that these differences are sufficient for the erection of a new species. Measurements and descriptions of these specimens are as follows.

(*3 females*).—L = 4.4 mm (4.0–4.8); a = 46.8 (44.0–48.4); b = 5.0 (4.8–5.2); c = 9.1 (7.5–10.7); V = 65% (63–67); buccal cavity = 75–80 × 52–58 μ; tail length = 0.49 mm (0.45–0.53).

(*2 males*).—L = 3.8–4.6 mm; a = 42.0–51.1; b = 4.7–5.2; c = 8.3–10.3; spicule length = 125–130 μ; buccal cavity = 70–75 × 50 μ; tail length = 0.45–0.46 mm; supplements = 15–16.

Diagnosis, female.—Differs from type form of species in body length and buccal cavity size. The buccal cavity walls are thicker and the teeth relatively



FIGS. 44–47. *Anatonchus* sp., near *alleni*. 44. Male tail enlarged. 45. Female. 46. Female head enlarged. 47. Male head enlarged.

larger in this variety. The caudal glands differ in shape and are more distinct than those of the type form of this species.

Diagnosis, male.—Differs from type form of the species in body length, in buccal cavity size, in length of spicule, and in size and shape of caudal glands. The spicules in this variety appear to be uneven in length. The gubernaculum may be bifid.

Habitat.—Orchard soil (walnuts).

Geographical distribution.—Santa Clara County, California, U.S.A.

Acknowledgments

The author is indebted to Dr. M. W. Allen, Professor of Nematology, University of California, California, for the use of the Californian collection upon which most of the present paper is based. Grateful thanks also go to Dr. A. D. Baker, Chairman, Nematology Section, Entomology Research Institute, Research Branch, Ottawa, to Dr. M. W. Allen and Professor G. Thorne, University of Wisconsin, Wisconsin, to Dr. A. C. Tarjan, University of Florida, and to Mr. B. E. Hopper, Nematology Section, Ottawa, for corrections and criticisms of the manuscript; and to Dr. S. A. Sher, University of California, for the loan of some of his nematode specimens.

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URCEOLARIA KARYODACTYLA N.SP. (CILIATA: PERITRICHA) FROM ISCHNOCHITON RUBER (L.) AT SAINT ANDREWS, NEW BRUNSWICK[1]

MARSHALL LAIRD[2]

Abstract

Urceolaria karyodactyla n.sp. occurs in the mantle cavity and on the ctenidia of its host, a circumpolar chiton. As seen in haematoxylin-stained slides it averages 65 μ in diameter and 60 μ in height, the diameter of the skeletal ring, which consists of 17–20 (usually 18) plates, averaging 23.5 μ. The macronucleus has an arm resembling a propeller blade on either side of a massive central body margined by several clubbed toe-like processes.

Trichodinid ciliates (Fam. Urceolariidae) have an aboral skeletal ring made up of interlocking denticles bearing radial projections. Members of the genus *Urceolaria* Stein differ from them in that this ring consists of obliquely positioned simple plates. They occur in the respiratory cavity of land snails, on fresh-water planarians, and in association with various marine invertebrates, especially intertidal annelids, limpets, and chitons. Although widely distributed, these ciliates have not previously been reported from Canadian coasts.

Materials and Methods

On June 28, 1958, 20 tortoise-shell limpets, *Acmaea testudinalis* (L.), and a red northern chiton, *Ischnochiton ruber* (L.), were taken from smooth boulders at the low-tide mark. The collecting locality was near the Biological Station of the Fisheries Research Board of Canada, Saint Andrews, New Brunswick (Passamaquoddy Bay, Bay of Fundy). All the limpets proved free from parasitic protozoa, but the circumpolar chiton harbored a few scyphidians (*Scyphidia ubiquita* Hirshfield—Peritricha: Sessilia: Scyphidiidae) and abundant urceolarians. The latter, the subject of this paper, are described from iron haematoxylin preparations over-destained for phase contrast examination. A Zeiss drawing apparatus was used in making the figures, and all averages are based on the measurements of 30 ciliates.

Descriptive Account

CILIATA: PERITRICHA: MOBILIA: URCEOLARIIDAE

Urceolaria karyodactyla n.sp. (Figs. 1–3)

Host: *Ischnochiton ruber* (L.) (referred by some authors to the genus *Trachydermon* or *Lepidochiton*).

Sites: Mantle cavity and ctenidia.

Taxonomic Data

Turban-shaped, height (39.5–71.0 μ; av., 60.0 μ) usually rather less than diameter (44.0–80.6 μ; av., 65.0 μ), ratio 3:5 to 6:5. Macronucleus elongate

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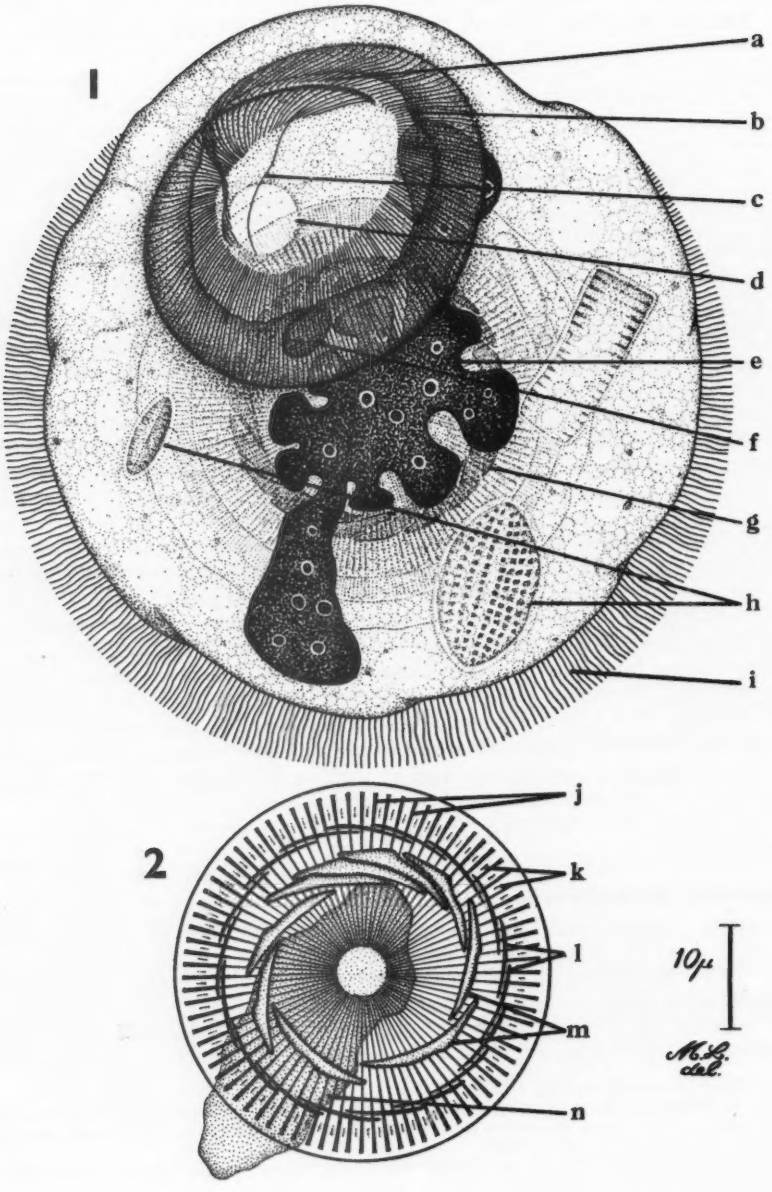
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., with financial assistance from the National Research Council of Canada and the Fisheries Research Board of Canada.

[2]Present address: Division of Environmental Sanitation, World Health Organization, Geneva, Switzerland.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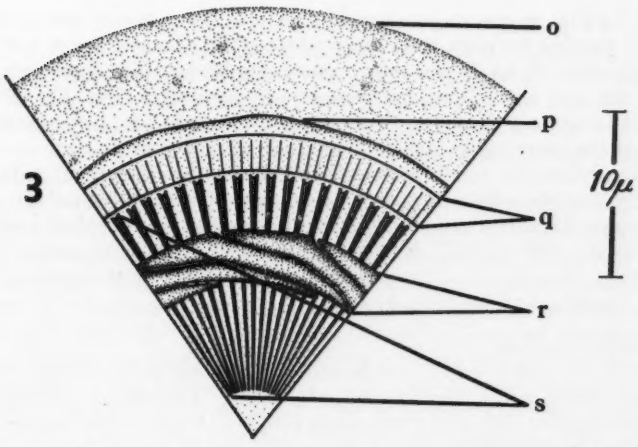

FIG. 3. *Urceolaria karyodactyla* n.sp., ×2450. Segment of mature individual, aboral view: *o*, edge of body; *p*, edge of velum; *q*, border membrane; *r*, skeletal ring; *s*, radial pin of striated membrane.

and of irregular outline (Fig. 2, *n*) following binary fission, rapid development thereafter leading to the formation of a massive central body with a long arm resembling a propeller blade on either side. Central body margined by several clubbed toe-like processes, separated by deep embayments (Fig. 1, *e*). Chromatin spherules, each in a clear vesicle, scattered throughout the whole structure. Micronucleus large (approx. 6 by 3 μ) and oval (Fig. 1, *f*), situated at one side of the central body of the macronucleus.

New skeletal plates of recently separated daughter individuals forming a ring 27.7–30.9 μ in diameter (Fig. 2, *l*), this ring contracting with growth of its plates and resorption of the inner ones derived from the parent (Fig. 2, *m*). Six such daughters had 18 plates in the outer ring and 9 in the inner, and two, 20 in the outer ring and 10 in the inner. Ring diameter in mature trophozoites, 19.1–27.2 μ (av., 23.5 μ) at the center of the plates, 21.2–30.1 μ (av., 26.3 μ) at their outermost limits. Thickness of ring, 2.3–3.7 μ (av., 2.8 μ). Skeletal plates (Figs. 2, *m*; 3, *r*) slightly curved, shouldered on the convex surface, and 10–13 μ long. Complement 17–20, 80% of the individuals examined having 18.

In aboral view, skeletal ring superposed on radial pins (Fig. 3, *s*) of a striated membrane 29.4–36.7 μ (av., 33.5 μ) in diameter. Radial pins numbering about 7 to the exposed outer surface of each skeletal plate, about 18 to the entire

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FIGS. 1 and 2. *Urceolaria karyodactyla* n.sp., × 1550. FIG. 1. Entire organism, viewed from adoral surface: *a*, adoral cilia; *b*, peristomal collar; *c*, cytopharynx; *d*, contractile vacuole; *e*, macronucleus; *f*, micronucleus; *g*, skeletal ring; *h*, ingested diatoms; *i*, marginal aboral cilia. FIG. 2. Adhesive disc following binary fission, aboral view: *j*, (parental) radial pins of striated membrane; *k*, newly forming radial pins; *l*, newly forming skeletal plates (normal complement); *m*, resorbing (parental) skeletal plates (half complement); *n*, macronucleus.

length of a plate, in mature trophozoites. After binary fission, new radial pins (Fig. 2, *k*) develop between those derived from the parent (Fig. 2, *j*) to restore the complement. A finely striated border membrane (Fig. 3, *q*) margins the adhesive disc just within the circumference of the velum (Fig. 3, *p*). Marginal cirri originating near the base of the latter obvious in life, but blending with the cilia of the inner aboral fringe (Fig. 1, *i*) after fixation.

Adorally, relatively small peristome surrounded by a deep collar (Fig. 1, *b*) apparently corresponding to the *Peristomsaum* of Zick (11) and the *collarette péristomienne* of Fauré-Fremiet (4). Circuit described by adoral cilia (Fig. 1, *a*), approximately 430° to 450°. Contractile vacuole (Fig. 1, *d*) adjacent to the cytopharynx (Fig. 1, *c*). The vacuolated cytoplasm with various food inclusions, notably diatoms (Fig. 1, *h*).

Systematic Position

Hirshfield (5) and Kahl (7) can be consulted for summarized data on species mentioned herein without individual referencing. *Urceolaria korschelti* Zick of Heligoland chitons (11) and Californian limpets (5, 6) ranges from 30 to 35 μ (11) or 40 μ (5) in diameter and 15 (5) to 25 μ (11) in height. Its macronucleus has a smooth to rugose central body, which an arm on either side joins at approximately right angles to a long, falciform lateral piece. The two lateral pieces are positioned one on either side of the ciliate, their convex surfaces outwards. Their tips are usually closer at one end, the entire macronucleus thus being A-shaped to H-shaped in adoral or aboral view. The macronucleus is of the same shape in *U. patellae* (Cuénot) of European limpets (1, 2), a species understood by Zick (11) and Hirshfield (5) to lack aboral cirri. Brouardel (1) has since shown that aboral cirri are in fact present in *U. patellae*, that somewhat greater size is the chief morphological feature distinguishing this organism (diameter 30–60 μ, usually about 45 μ) from *U. korschelti*, and that the latter may have to be relegated to the status of a race or subspecies of the former. *U. cellanae* Suzuki of Japanese limpets (10) also has an A- to H-shaped macronucleus, and stands very close to *U. patellae* as redescribed by Brouardel. Although the skeletal ring of the former species has fewer plates (13–16, usually 14) than average examples of the latter, the overall range for *U. patellae* is 12–25 (1). Experience with other urceolariid genera suggests that these are but slender grounds for specific differentiation. Two further urceolarians likewise differing from the Canadian one in having an A- to H-shaped macronucleus are harbored by annelids. These are *U. serpularum* (Fabre-Domergue) from Europe (3), and *U. urechi* (Noble) from California (8) and Japan (10).

The macronucleus is C-shaped in *U. mitra* (Siebold) and *U. ozakii* Suzuki (10), and L-shaped or reniform in *U. synaptae* Cuénot. Hirshfield (5), rejecting *U. terebellidis* (Precht) as a nomen nudum, interpreted the admittedly confusing description (9) to signify that the macronucleus is lobate. He apparently overlooked Precht's Fig. 4*e* (ref. 9), in which this structure is illustrated as C-shaped. The macronucleus is said to be discoidal in *U. scoloplontis* (Precht), and lenticular to ovoidal in *U. paradoxa* (Claparède and Lachmann). It is lobate in *U. karyolobia* Hirshfield (5), the only species warranting detailed comparison with mine.

Urceolaria karyolobia, which occurs on various limpets in California, is smaller than the present ciliate, ranging from 45 to 60 μ in diameter and from 20 to 35 μ in height. It has 16–22 skeletal plates 8–10 μ long, there being only eight radial pins per plate. The macronucleus is of irregular outline, upward-projecting marginal lobes forming a cup-like structure within which lies a small micronucleus (5). These features clearly distinguish *U. karyolobia* from the species described herein.

The latter is accordingly regarded as new. Like other species of *Urceolaria* relegated by some authors to a distinct genus (3) or at all event subgenus (4), *Leiotrocha* Fabre-Domergue, it has marginal aboral cirri. Brouardel (1) has rightly contended that although these organelles are easily overlooked, the existence of urceolarians altogether lacking them has yet to be demonstrated, and that therefore only one subgenus (*Urceolaria*) can be validated. The ciliate is designated *Urceolaria karyodactyla* n.sp. in reference to the lateral toe-like processes (Gr., *daktylos*, a finger, toe) of the central body of its macronucleus (Gr., *karyon*, a nut, nucleus). The type slide has been deposited in the collection of the U.S. National Museum, Washington, D.C. (USNM No. 23677).

Acknowledgments

Friendly co-operation was extended by Dr. J. L. Hart, Director of the Biological Station, Fisheries Research Board of Canada, Saint Andrews, New Brunswick, and various members of his staff, during the investigations with which this contribution deals.

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TRICHODINIDS AND OTHER PARASITIC PROTOZOA FROM THE INTERTIDAL ZONE AT NANAIMO, VANCOUVER ISLAND[1]

MARSHALL LAIRD[2]

Abstract

Haemogregarina bigemina Laveran and Mesnil (Sporozoa: Coccidia) is reported from the padded sculpin, *Artedius fenestralis* Jordan and Gilbert, and *Eupoterion pennix* MacLennan and Connell (Ciliata: Holotricha) and *Scyphidia ubiquita* Hirshfield (Ciliata: Peritricha) from the limpet, *Acmaea cassis* Eschscholtz. These are new host and locality records. Two trichodinids (Ciliata: Peritricha) are described from *A. fenestralis*. *Trichodina vancouverense* n. sp. has 16 to 25 (av., 22) denticles with blades averaging 3.0 μ in height, the striated membrane extending for about the same distance beyond them. The diameter of the denticulate ring at the outer limits of the denticles corresponds with the inner diameter of the macronucleus. *T. decipiens* n. sp. has 23 to 34 (av., 28) denticles, the blades of which average 6.5 μ in height and almost reach the levels of the outer edges of the striated membrane and macronucleus.

Introduction

A modern work on invertebrates of California's intertidal zone (13) devotes only part of one of its 446 pages to the phylum Protozoa. It thereby underrates both a highly important aspect of the zone and this particular State's relevant contributions. Not only is an acquaintance with protozoology basic to the understanding of food chains, but seashores offer rewarding fields for research into the ecology of parasitic as well as free-living protozoons. Californian investigations on parasitological aspects of the subject have furnished valuable papers on Myxosporidia from tide pool fishes (17, 18), on Peritricha from limpets and turbans (8, 9), and on *Licnophora* (1, 28), *Trichochona* (16), and other ciliates (15, 20, 28), from various marine invertebrates. Current studies of general host–parasite relationships in the euryhaline zone (19) should be mentioned too.

Data on protozoan parasitism in coastal waters diminish progressively with movement northward. Several papers by Kozloff on molluscan Holotricha comprise a significant contribution from Portland, Oregon, while there has been recent work on sea urchin ciliates from Washington (2). But on the Canadian Pacific seaboard, although considerable attention has been paid to free-living marine Protozoa (33), parasitic species have been virtually ignored.

Berger (2) has commented on the marked revival of interest in the ciliates of echinoderms in the Pacific's northwestern sector, and this interest extends to other protozoan groups too. To keep abreast of developments there, intensified studies are called for all along the Pacific coast of North America on such readily available though locally neglected organisms as the cnidosporidians and blood parasites of marine fishes, and the ciliates associated with

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[2]Present address: Division of Environmental Sanitation, World Health Organization, Geneva, Switzerland.

so many sea animals.

On October 30, 1958, a visit to the Biological Station of the Fisheries Research Board of Canada at Nanaimo, Vancouver Island, B.C., provided some tangible evidence of the rich field awaiting investigation there.

Methods and Materials

Ten 40- to 65-mm specimens of the padded sculpin, *Artedius fenestralis* Jordan and Gilbert, were obtained by poisoning an intertidal rock pool with 5% liquid rotenone. Three crabs, *Hemigrapsus nudus* (Dana), were captured from beneath marginal boulders, and several shellfish (eight limpets, *Acmaea cassis* Eschscholtz, and four mussels, *Mytilus edulis* L.) were taken from rocks near the mid-tide mark.

Blood films were made from the heart of each fish, and fresh scrapings from the gills and intestine of every specimen were examined by means of a McArthur field microscope with an attached, battery-powered lamp outfit—a pocket-sized piece of equipment invaluable for such surveys. Protozoa were not found in the crabs or mussels, but the fish and limpets were parasitized as reported in the following section. Smears were made from all positive specimens on albuminized 18-mm-square cover slips (No. 1). These were fixed for half an hour in Davis' (4) modification of Worcester's fluid—75 cc of a saturated solution of mercuric chloride, 20 cc formalin (formaldehyde 40%), and 5 cc glacial acetic acid. They were then washed and transferred to iodized 70% alcohol in 5×2.5-cm clear plastic tubes, the stoppers being secured with cellulose tape for transportation. On my return to Montreal, the blood films were treated with Giemsa's stain in the usual fashion. The other material was stained with iron haematoxylin, destained in a saturated aqueous solution of picric acid, and mounted in Permount (Fisher Scientific Company). Phase contrast proved useful in revealing details of understained parts of ciliates. A Zeiss drawing apparatus was used in the preparation of the figures, and, unless otherwise stated, all averages are based on the measurements of 30 specimens of each organism.

Systematic Account

SPOROZOA: COCCIDIA: ADELEIDEA: HAEMOGREGARINIDAE
(higher taxa, those of Kudo, ref. 10)

Haemogregarina bigemina Laveran and Mesnil, 1901
Host: *Artedius fenestralis* Jordan and Gilbert.
Site: Heart blood.

Films from two fish proved lightly infected with haemogregarines. A few erythrocytes contained paired vermicules, 12 of which measured 10.1–14.1 μ by 1.0–1.9 μ (av., 12.3×1.4 μ). The broader end of the organism is rounded, the other tapering to a tail towards which the nucleus is located. This parasite conforms to the description of *H. bigemina* from various hosts, which include blennies and other tide pool fish in the South Pacific (11, 12).

CILIATA: HOLOTRICHA: THIGMOTRICHA: HEMISPEIRIDAE
(classification adopted by Kudo, ref. 10)
(THIGMOTRICHIDA, according to Corliss, ref. 3)

Eupoterion pernix MacLennan and Connell, 1931

Host: *Acmaea cassis* Eschscholtz.

Site: Intestine.

Thigmotrichous ciliates were plentiful in the intestinal contents of five limpets. When first transferred to the microscope stage, they moved rapidly and in a straight line with a clockwise rotation. Progressional movements soon ceased, and the organisms were seen to be laterally flattened and approximately oval, but dorsally reniform save for a distinct point anteriorly. Peristomal cilia, stouter and about twice as long as the locomotory ones, were prominent on the mid-ventral surface.

Only seven individuals remained on the cover slips following fixation and staining. They measure 32.3–56.1 μ by 22.0–38.6 μ (av., 46.3×31.2 μ), and have an almost spherical macronucleus, 10.0–13.5 μ in diameter, towards the anterior extremity. Just in front of it is a spherical micronucleus some 3 μ in diameter. There are upwards of 40 anteroposterior ciliary rows (the exact number cannot be made out), and six rows of peristomal cilia.

These data fit the description of *Eupoterion pernix*, originally found in *Acmaea persona* Eschscholtz at San Francisco (15), and since recorded from six species of limpets at Los Angeles (9).

CILIATA: PERITRICHA: SESSILIA: SCYPHIDIIDAE
(PERITRICHIDA: SESSILINA according to Corliss, ref. 3)

Scyphidia (*Gerda*) *ubiquita* Hirshfield, 1949

Host: *Acmaea cassis* Eschscholtz.

Site: Gill lamellae.

All eight limpets were parasitized, broad-based scyphidians being firmly attached to their gill lamellae by means of a prominent scopula. The length-to-breadth ratio of fully extended live specimens was rather more than 3:1, the bullet-shaped peristomal disc being a prominent feature. Most individuals in stained preparations are partly or fully contracted, the former measuring 52.1–70.0 μ by 26.3–44.5 μ (av., for 16, 58.6×34.8 μ), and the latter, 30.8–48.9 μ by 24.1–40.4 μ (av., for 20, 42.8×31.5 μ).

The sausage-shaped macronucleus is longitudinal y al gned in extended examples and tilted or more or less parallel with the base in fully contracted ones. Its length is about two-fifths that of the partly contracted body. The micronucleus is large (av., 5.6×3.6 μ) and lenticular. Although usually located just beneath the peristomal disc, it is sometimes deep within the cytoplasm. There is a long and prominent cytopharynx, terminating at a food vacuole which, in partly contracted individuals, is in the posterior third of the body. A thick and rugose scopula is broadly based on the body proper, adjacent parts of the pellicle of which may be thrown into folds on contraction. The pellicular annuli, difficult enough to discern by bright-field illumination in life, are not apparent in haematoxylin-stained specimens.

Some individuals have a thickened pellicle, their cytoplasm being retracted about the macronucleus. These agree with Hirshfield's (8) "resistant stages" of *Scyphidia ubiquita* from turban shells and limpets (including six species of *Acmaea*) in southern California. The Nanaimo scyphidians compare so closely with this species in every respect, including the distinctive overlapping

of the peristomal ridges of fully contracted individuals (see ref. 8, Fig. 5C), that there can be no justification for not referring them to it.

CILIATA: PERITRICHA: MOBILIA: URCEOLARIIDAE
(PERITRICHIDA: MOBILINA according to Corliss, ref. 3)

The gills of all 10 sculpins bore abundant urceolariids of two sharply distinct morphological types. No cirri are recognizable in the fixed and stained specimens, but all of them have an aboral ring of denticles with both outer blades and inner rays, and the adoral spiral performs a circuit of about 450°. They are thus referable to *Trichodina* Ehrenberg. Most of Lom's (14) proposals for uniform specific criteria are adopted herein, and two further characters are mentioned. These are the diameters at the innermost and outermost limits of the denticles. The over-all diameter of the denticles at the outermost limits of their blades was also employed by Fauré-Fremiet (7).

Despite the well-marked characters exhibited by these ciliates, those authors describing them have too often confined themselves to criteria of little more than generic significance. Unfortunately, numerous poorly described species are just sufficiently well characterized to disallow rejection of their names as nomina nuda. Pending redescription of such trichodinids, it is not possible to prepare serviceable keys.

As evidence of the taxonomic uncertainties among species of *Trichodina*, although these organisms have an oval or rounded macronucleus immediately after they undergo binary fission (11), the structure becomes C- or horseshoe-shaped by maturity. This is so general a rule that the status of *T. ovonucleata* Raabe, 1958, should be critically re-examined. Raabe (23) stressed that this ciliate, the chief specific feature of which is an obovoid macronucleus, is one of the smallest members of its genus, and of course recent products of binary fission are relatively small too. The same host, an Adriatic blenny, invariably carried a morphologically distinct trichodinid that Raabe (23) named *T. rectuncinata*. Do these protozoons, despite their macronuclear and denticular differences, really constitute distinct species? And, for that matter, are other similarly differentiated pairs—e.g., *T. spheroidesi* and *T. halli* (21), *T. halichoeri* and *T. microrbis* (30), *T. parabranchicola* and *T. multidentis* (11), and that described herein—found by various investigators in apparent double infections, really distinct? Is it unreasonable to hypothesize that some at least of their differences may be due to sexual dimorphism? Regrettably, with present knowledge the taxonomist can do no more than admit the possibility and continue to employ what might eventually prove invalid criteria.

Much of the relevant literature consists of arbitrary personal impressions based on limited material and often biased by unwarranted assumptions of strict host specificity. The situation is further complicated by the fact that

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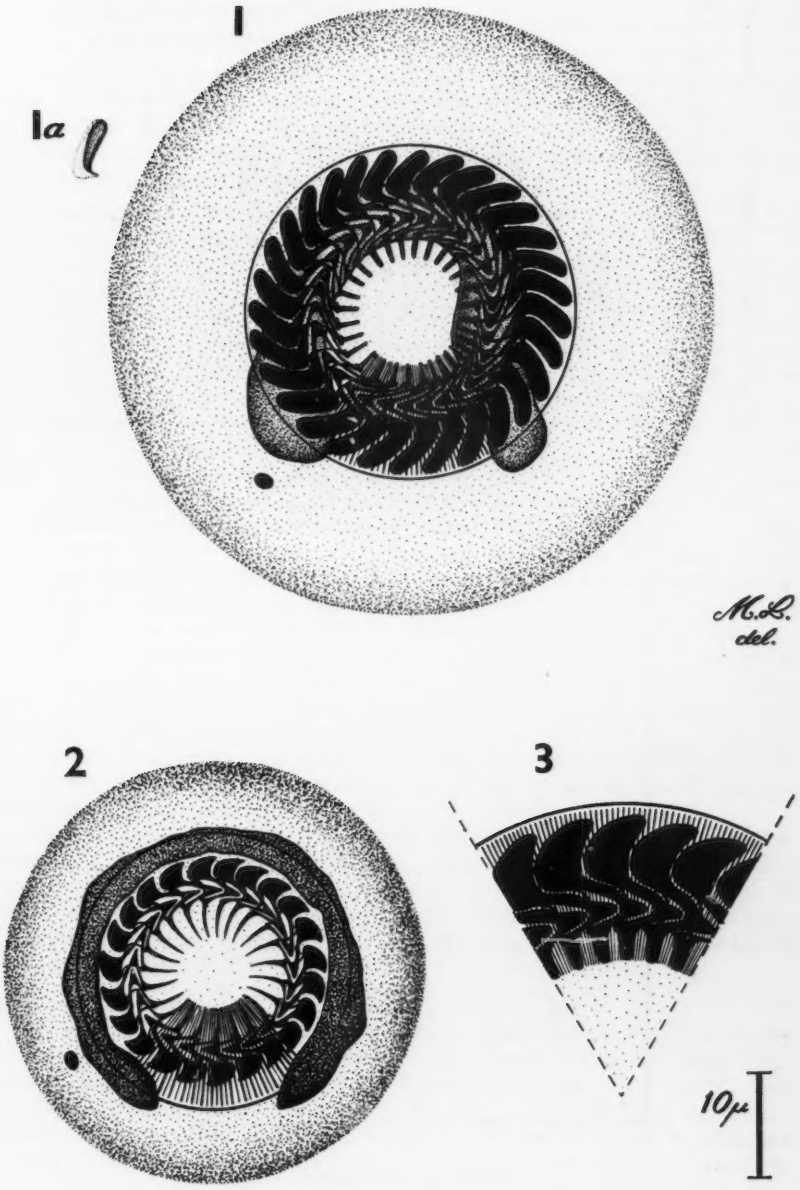
FIGS. 1–3. Semidiagrammatic aboral views, ×1550. FIGS. 1 and 2. *Trichodina decipiens* n. sp. and *T vancouverense* n. sp., respectively, showing nuclei, denticulate ring, outer edge of striated membrane, a few only of the radial pins, and body outline. FIG. 1*a*. Illusory club-shaped appearance of denticular blade of *T. decipiens* n. sp., caused by the more intense staining reaction of the zone nearest the hook. FIG. 3. Segment of one of the largest individuals of *T. decipiens* n. sp., showing denticles, radial pins, and outer edge of striated membrane.


1
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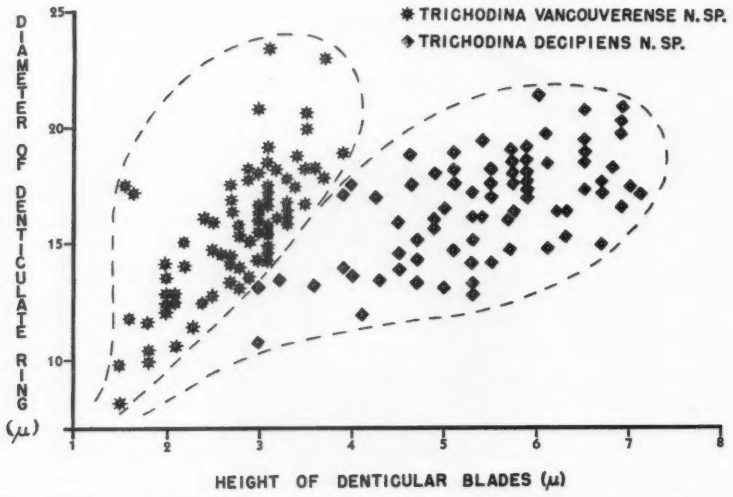

FIG. 4. Biometrical differentiation of *Trichodina vancouverense* n. sp. and *T. decipiens* n. sp., based on the height of the denticular blades and the diameter of the denticulate ring at the center of the cones.

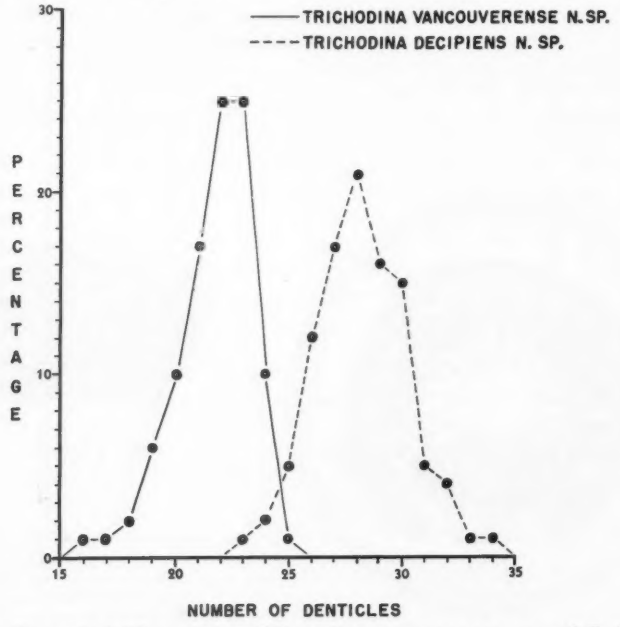

FIG. 5. Biometrical differentiation of *Trichodina vancouverense* n. sp. and *T. decipiens* n. sp., based on the percentage of individuals of each species exhibiting particular numbers of denticles.

protozoologists have been conspicuously less conscientious than their colleagues in other fields of zoology, in the matter of depositing type material in museums. However, while future zoogeographical and experimental work may well establish that there are, after all, relatively few (although widely distributed) species of *Trichodina*, it remains necessary, for the present, to establish yet more species when, despite careful comparisons, existing descriptions fail to accommodate new material.

Trichodina vancouverense n. sp. (Figs. 2, 4, 5)

Host: *Artedius fenestralis* Jordan and Gilbert.

Site: Branchiae.

Taxonomic Data

Turban-shaped, height less than diameter, ratio 1:2 to 4:5. Binary fission commonest among individuals towards the lower limit of the size range, macronucleus of youngest daughter forms oval or approximately so, thereafter undergoing rapid elongation and becoming horseshoe-shaped (Fig. 2). During the last phase of this process, micronucleus sometimes in Dogiel's (5, 14) $-y^1$ or $-y$ positions, but, at maturity, usually in his $+y$ position (Fig. 2), that is, on the left side of the left arm of the macronucleus, a short distance back from its tip, as viewed from the aboral aspect.

New denticular plates of recently separated daughter individuals forming a ring 14.9–20.4 μ in diameter. Ring contracting with the resorption of the denticles derived from the parent, its blades, cones, and rays becoming recognizable meanwhile. Its diameter at center of cones, 8.0–23.4 μ (Fig. 4). Of 75 mature denticulate rings measured, 65 fell within the range 11–19 μ (av., 16 μ). Recent products of binary fission with 9–11 resorbing denticles derived from the parent in an inner ring 6.3–9.9 μ in diameter, twice this number (± 2) in the new outer one. Denticular number 16–25 (av., 22), blades broad, strongly recurved and sharply pointed terminally, and rays long, retrorse, thin, and sharp. Average measurements of fully developed denticle from outermost point of blade to innermost one of ray: blade (= hook), 3.0 μ; cone (= central part), 1.6 μ; ray (= spike), 4.3 μ.

Striated membrane (= striated band) with seven or eight radial pins (= striae) per denticle; over-all diameter, 26.8–33.8 μ (av., 30.6 μ). Other significant diameters: body, 30.9–58.7 μ (av., 44.2 μ); outermost limits of blades, 21.1–26.5 μ (av., 23.6 μ); innermost limits of rays, 4.3–8.0 μ (av., 5.9 μ); outer edge of macronucleus, 23.3–33.5 μ (av., 30.0 μ); inner edge of macronucleus, 19.8–27.1 μ (av., 23.8 μ).

Systematic Position

This species bears comparison with several others having denticles of the *pediculus* type (24), and parasitizing marine fishes. Some of these (e.g. *Trichodina scorpaenae* Robin, 1879, *T. labrorum* Chatton, 1910, and *T. clini* Fantham, 1930), although close to the present one in size and denticular number, require careful redescription before new material can be assigned to them. The hosts of *T. branchicola* Tripathi, 1948 (32), include a member of the Cottidae (the family to which *Artedius fenestralis* belongs). However, although this British species has from 20 to 26 denticles (av., 22) it is not known to have fewer than 20, there are usually only six radial pins per

denticle, and the denticles themselves have shorter blades (2.3 μ) and rays (3.2 μ) than those of the Nanaimo species; it also differs from the latter in other details, including the fact that its micronucleus is located in a pocket on the outer edge of the macronucleus and can be seen only in sections. *T. cottidarum* Dogiel, 1948 (5), is known from several cottids and other fishes in the White Sea, Sea of Japan (5), Barents Sea (22), and Baltic (25, 27, 29). In this species, of which several forms have been described, the micronucleus is typically in Dogiel's (5) $-y$ position (that is, in line with the prolongation of the left arm of the macronucleus, in aboral view). Its denticular blades measure 3.5–4.5 μ (av., 4 μ) from the center of the cones, and its rays 4–5 μ (av., 4.5 μ). These lengths average 3.8 μ and 5.1 μ, respectively, in my species, the rays of which are not only proportionately longer than the blades in *T. cottidarum*, but are needle-like, not bluntly pointed like those of the latter. In their general appearance, the denticles of the present trichodinid, in fact, much resemble those of an otherwise dissimilar parasite from American fresh-water fishes, *T. discoidea* Davis, 1947 (4). Finally, *T. cottidarum* has a flattened (25), not a turban-shaped, body, and in aboral view the denticles are superposed on the micronucleus (ref. 25, Pl. VII). The outermost limits of the blades and the inner edge of the macronucleus are of similar diameter in the species under discussion, the denticles of which thus usually appear framed within the macronucleus (Fig. 2).

More data and abundant mounted specimens are available for a related trichodinid of New Zealand intertidal zone fishes, *Trichodina parabranchicola* Laird, 1953 (11). This species has from 16 to 26 (av., 22) denticles, the diameter of its denticulate ring ranging from 9.5 to 30.0 μ (av., 17.5 μ). Its denticular blades and rays both average 4.0 μ in length, a feature distinguishing it from the Nanaimo parasite, which also has a rather smaller denticulate ring. The organisms further differ from one another in two features not apparent from the published account of *T. parabranchicola*. In the latter, the cones and blades of the denticles are usually superposed on the macronucleus in aboral view, as in *T. cottidarum*. The second feature is one advocated by Stryjecka-Trembaczowska (29), the angle enclosed by radii from the center of the adhesive disc to the two extremities of the macronucleus. This angle is approximately 75° in *T. parabranchicola*, but only 45° in the Nanaimo parasite.

The present species is accordingly regarded as allied to, but on present evidence distinct from, *T. branchicola*, *T. parabranchicola*, and the *T. cottidarum* complex. It is designated *Trichodina vancouverense* n. sp., in recognition of its discovery in the Vancouver Natural Region of Canada (that is, the southwest corner of the Province of British Columbia, so styled as one of the 20 natural regions of Canada (31)). The type slide and a paratype have been deposited in the collections of the United States National Museum, Washington D.C. (USNM 23678 and 23679).

Trichodina decipiens n. sp. (Figs. 1, 1*a*, 3–5)

Host: *Artedius fenestralis* Jordan and Gilbert.

Site: Branchiae.

Taxonomic Data

Shaped like a truncated cone capped with the low dome of the peristome,

height equal to or somewhat greater than diameter, ratio 1:1 to 5:4. Binary fission commonest among individuals towards the lower limit of the size range, macronucleus of youngest daughters oval or approximately so, that of mature specimens thick and C-shaped (Fig. 1). At maturity, micronucleus usually in Dogiel's (5) $-y$ position (that is, in line with the prolongation of the left arm of the macronucleus, in aboral view).

Denticular plates of recently separated daughter individuals forming a ring 18.2–27.2 μ in diameter, contraction and denticular growth as for the preceding species. Ring diameter[3] at center of cones, 10.6–21.3 μ (Fig. 4); 65 of the 75 measured, within the range 13–19 μ (av., 17 μ). Recent products of binary fission with from 14 to 18 (av., 15) resorbing denticles derived from the parent in an inner ring 7.8–10.1 μ in diameter, twice this number (± 2) in the new outer one. Denticular number 23–34 (av., 28), blades relatively narrow, strongly recurved, and terminally rounded, and rays short, straight, thick, and bluntly rounded. Average measurements of fully developed denticle: blade, 6.5 μ high; cone, 3.5 μ thick; ray, 2.5 μ long.

However, in average-sized individuals (Fig. 1) the blade is usually between 4 and 7 μ high (av., 5.7 μ) (Fig. 4). The higher over-all figure of 6.5 μ is due to the fact that in some unusually large specimens (Fig. 3), several of which were included in the sample, the blade measured up to 10 μ. In such large ciliates, the blade is relatively broader than in average-sized ones which afford less room for its lateral growth (Fig. 1). Failure of the thinner distal part of the blade to stain with any intensity often gives the structure an illusory club-shaped appearance (Fig. 1*a*).

Striated membrane with (usually) 7–9 radial pins per denticle, as many as 13 in largest specimens (Fig. 3); over-all diameter, 30.8–59.2 μ (av., 34.6 μ). Other significant diameters: body, 41.3–98.6 μ (av., 60.8 μ); outermost limits of blades, 28.2–58.9 μ (av., 33.5 μ); innermost limits of rays, 9.8–27.1 μ (av., 12.6 μ); outer edge of macronucleus, 29.0–49.9 μ (av., 34.8 μ); inner edge of macronucleus, 14.2–40.8 μ (av., 23.2 μ).

Systematic Position

This organism is easily distinguished from *T. vancouverense* by biometrical methods, its denticular blades at given ring diameters always being higher, and usually much higher, than those of the latter species (Fig. 4); and there being only a small overlap in denticular number (Fig. 5). It has a larger and more cylindrical body than its associate, from which it further differs in that its denticular rays are thick and blunt, the blades being two to three times their length and reaching almost to the levels of the outer edges of the macronucleus and striated membrane (Figs. 1, 3).

Inadequately described species with a comparable denticular number include *T. bidentata* Fabre-Domergue, 1888 (30 denticles), *T. blennii* Fantham, 1930 (24–32), *T. mugilis* Fantham, 1930 (32), and *T. chelidonichthyos* Fantham, 1930 (30). Fantham's three species, from South African marine fishes, are

[3]In the exceptionally large individuals mentioned in the following paragraph, the diameter of the denticulate ring at the center of the cones ranged up to 38.8 μ. No evidence was obtained that these belong to a separate species, their morphology not being incompatible with continued growth of the common average-sized examples. Not without misgivings, the large ciliates are thus viewed as giant forms of *T. decipiens* n. sp.

broader than high; the range of their adhesive disc diameters falls short of the lower limit of the diameter of the striated membrane of the present ciliate, and the best-described of them, *T. blennii*, is said to have only five radial pins per denticle (6).

T. spheroidesi Padnos and Nigrelli, 1942 (21), has from 21 to 31 denticles, the diameter of the ring (14–22 μ) comparing quite closely with that of the species under discussion. The two organisms are of quite similar size, and both have the micronucleus in the $-y$ position. Unfortunately, it is not possible to make out the shape of the denticles from the description and (side elevation) figure presented by Padnos and Nigrelli, but the fact that the rays are referred to as "slender" and appear from the figure to be long and pointed preclude referring the present species to this one. *T. halli* Padnos and Nigrelli, 1942 (21), with from 26 to 34 denticles, is a much larger species in which the ring diameter ranges from 30 to 54 μ. The denticular blades closely resemble those of the present species, but the rays taper to a sharp point, and both they and the blades appear from text figure 2B of Padnos and Nigrelli (21) to measure some 6–8 μ.

Trichodina microrbis Suzuki, 1950, from a Japanese marine fish, has only 19–23 (usually 22 or 23) denticles, but from Suzuki's fine illustration (30, Pl. 6, Fig. 1) the form of these much resembles that of average-sized specimens of the Nanaimo ciliate. Both have relatively thick and intensely staining denticular cones, and short, blunt rays (it is clear from Suzuki's figure that he prepared it from an insufficiently flattened animal, the denticles thus being viewed obliquely). *T.* (*Cyclochaeta*) *halichoeri* Suzuki, 1950, from the same host as *T. microrbis*, is a larger organism quite comparable in size and shape with the present one. It has 25–29 denticles, in which, however, the blade is scimitar-shaped and equalled in length by the ray.

Trichodina multidentis[4] Laird, 1953 (11), from various New Zealand intertidal fishes, has from 23 to 45 denticles (usually 30–37, av., 32). It has more denticles, blunter and relatively higher blades, and thicker rays, than its associate, *T. parabranchicola*, these differences paralleling those between the present species and *T. vancouverense* n. sp. In the original description of *T. multidentis*, the blades and rays were measured from the center of the cone, and were stated to average some 4.5 μ each. Fresh measurements from some of the paratype slides give the following averages: blade, 3.7 μ high; cone, 2.0 μ thick; ray, 3.6 μ long. The proportions of the denticular elements are thus quite different in the two ciliates. *T. multidentis* further differs from its Canadian counterpart in the much greater diameter of the denticulate ring (34.8 μ) of average-sized individuals, and the fact that its blades fall well short of the outer diameter of the striated membrane.

Trichodina dohrni Reichenbach-Klinke, 1956 (26), from a marine fish of the Gulf of Naples, combining 29–32 denticles with a ring diameter of 32–38 μ,

[4]This name was proposed in ignorance of Suzuki's (30) use of the same roots 3 years previously, for his (*Cyclochaeta*) = *Trichodina multidentata* of Japanese tadpoles. However, the termination *dentis* is substantive while *dentata* is adjectival. Two names so differing are not regarded as homonymous under Article 35 of the International Rules of Zoological Nomenclature (Bull. Zool. Nomenclature, 4, 242 (1950)), although Concl. 17 on p. 296 of this authority recommends that such names should not be deliberately chosen for species of the same genus or related genera. Suzuki's paper has escaped the notice of European authors too.

bears comparison in these respects with *T. halli*. Unlike the latter species but like the present one it has shorter denticular rays than blades, the former ranging from 3.6 to 3.9 μ in length and the latter from 3.8 to 6.0 μ. However, the macronucleus of *T. dohrni* is relatively small, lying completely within the space enclosed by the innermost limits of the denticular rays; a further point of difference from the present species is that the denticular blades are separated from the outer limit of the striated nembrane by an interval approximately equivalent to the length of the blades themselves.

The chief feature said to distinguish *T. rectuncinata* Raabe, 1958 (23), from other members of its genus is the shape of the blades. From Raabe's figure 3*b*, these much resemble those of the smaller specimens of the present species, differing chiefly in their more-pointed shape, which, together with the backward-pointing appearance of the rays, suggests that the figure may have been prepared from an inadequately flattened example. Such an appearance is presented by many of the specimens on my slides, and the two organisms are comparable in some other respects. Thus, in *T. rectuncinata* the denticular blades are from two to three times the length of the rays, they almost reach the outer edge of the striated membrane, the denticular number ranges from 20 to 33, and the micronucleus is in the $-y$ position. Nevertheless, the two cannot be regarded as conspecific from the available data. Besides the need for clarity with respect to the shape of its denticles, the Adriatic species usually has only 22–24 of these, with six or seven radial pins to each.

Differing as it does from *T. vancouverense* n. sp. with which it is associated, and from other members of the genus as indicated above, the species under consideration is designated *Trichodina decipiens* n. sp. The specific name is derived from the Latin *decipere*, to deceive, and refers to the illusory club-shaped appearance frequently manifested by the denticular blades. The type slide and a paratype have been deposited in the collections of the United States National Museum (USNM Nos. 23680 and 23681).

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THE INFLUENCE OF CLIMATE ON METABOLIC AND THERMAL RESPONSES OF INFANT CARIBOU[1]

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Abstract

Metabolic and thermal responses of infant caribou to climate were measured during the June calving period on the barrens in the area of Mosquito Lake and Beverly Lake, N.W.T. It was found that temperature regulation was well established at birth and that the calves were very sensitive metabolically to cold, wind, and precipitation. The metabolic rate was doubled by a lowering of temperature to about 0° C, but cold combined with wind and precipitation elevated the metabolic rate to over five times the resting value. Calves which were exposed without protection to such conditions eventually became hypothermic and died. Weather conditions during storms on the barrens are sufficiently severe to produce some mortality in animals exposed without protection. The possibilities for prediction of mortality from wind chill values and estimated fur heat loss are discussed.

Caribou are exposed to the exigencies of cold, wind, and precipitation just after birth. Newborn calves follow the female about regardless of severe weather conditions that seem unfavorable to their survival. Field evidence (1) has suggested that mortality may increase during years of severe climate stress, but no physiological evidence indicating the tolerance of the animals to the prevailing climatic conditions has been provided.

To meet this need it is necessary to have field information on climatic conditions and mortality on the calving ground, and to obtain physiological data on (1) tolerance of calves to the weather conditions and (2) metabolic and thermal responses of the animals to these conditions. In the present study, information is provided on the cold tolerance and on thermal responses of newborn and of a 9-month-old calf. The metabolic responses have been correlated with wind chill index and with heat loss through the fur to integrate the effects of temperature and wind on newborn caribou.

Methods

Test Conditions

Tests of metabolic responses and of cold tolerance were carried out at a field laboratory at Mosquito Lake, N.W.T., in June 1958. Further studies of cold tolerance were carried out on the calving grounds in the vicinity of Beverly Lake, N.W.T., in June of the following year. At Mosquito Lake, continuous records were kept of the environmental temperature, humidity, wind velocity, rainfall, and sunlight. At the field camp at Beverly Lake only temperature was recorded continuously. Other factors were recorded periodically.

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For studies at Mosquito Lake in 1958, 10 young animals were captured soon after birth (body weight 11–12 lb) in the Sprucegrove Lake – Beverly Lake area, and within 48 hours were flown 175 miles south to the laboratory at Mosquito Lake. Immediately thereafter they were kept in a snow fence corral without forage and fed on a milk diet* every 4 hours. Tests on these animals began immediately or on the following day and were completed after 2 to 5 days. After the tests the calves were allowed to forage around camp. All six male calves remained in good health throughout the period of captivity, but one female died shortly after capture and the other female, apparently suffering from a respiratory ailment, did not feed or gain weight normally.

In 1959, nine calves were captured at Beverly Lake within 2 hours of birth and were flown the short distance back to camp. These animals were kept in a manner similar to those kept at Mosquito Lake except that the camp was in a more exposed location. They were initially given the same milk diet but this was later changed to one of diluted canned milk only. The calves appeared in good condition except for diarrhea in some animals. The death of one animal, which was being tested under moderate conditions, may be attributed partly to diarrhea. The diarrhea was not altered noticeably by the change in diet.

Metabolism and Body Temperature

During the measurements of metabolism and body temperatures, the animals were secured by a neck stanchion and a loosely fitting canvas sling, which held them in a standing position. They were tested under four general environmental conditions: (1) no wind, inside the laboratory building at 10° to 20° C, (2) no wind, in a tent at approximately 0° C, (3) outside, exposed to wind at approximately 0° C, and (4) outside, exposed to wind when the fur was soaked with water. All tests were conducted in the shade.

During each test a rubber hood with a plastic nosepiece was placed over the head of the animal. The nosepiece was fitted with a valve which directed the expired air through a large diameter hose into a 40-liter Douglas bag. The oxygen consumption was calculated from the respiratory minute volume and from the O_2 and CO_2 content of the expired gas as measured by a Scholander $\frac{1}{2}$-cc analyzer (3). Oxygen consumption was normalized for differences in weight by expressing it as a function of body weight to the 0.75 power (Brody, 2) and converted to heat production from the appropriate R.Q. values.

Rectal temperatures were taken by means of thermocouples before and after one to three bag samples were obtained. Skin temperatures were also obtained by thermocouple probes placed under the fur on four locations on the back and right side of the body and on one location on the hind foot. Most of the animals were quiet during the actual tests. Measurements during which struggling took place were excluded.

Cold Resistance

Cold resistance tests were carried out on four calves at Mosquito Lake in 1958 and on eight calves at Beverly Lake in 1959. In the Mosquito Lake tests two calves, 3 days old, were tested in a snow fence corral on top of an esker (June 6–7) and two calves, less than a day old, were tethered by a rope in the

*Per quart: 101 g dried cream, 471 g evaporated whole milk, 490 g water, 11 g mineral mix.

same area but not protected by a corral (June 9–10). The mean temperature and wind conditions are shown in Tables I to IV. Driving snow was present in both tests.

At Beverly Lake (Table IV) three calves were exposed to moderate winds at near freezing temperatures for 12 hours (June 8), one calf was tested a few minutes after birth while still wet (June 13), and four calves were tested on June 15 under conditions of low temperature, wind, and rain. Body temperatures were recorded using a portable thermistor bridge (Yellow-Springs Thermistemp). During the tests the animals were tethered by a rope. Some were blindfolded to prevent struggling.

Tests on a 9-Month Calf

Tests were carried out on one of the Mosquito Lake calves that was flown to Ottawa in late June 1959 and maintained at the Central Experimental Farm. The animal was tested in March to assess its responses to cold at a time equivalent to its first winter exposure. At this time it weighed 70 lb but it was not considered to be as large as a normal wild 9-month calf.

Measurements of the oxygen consumption were carried out at 25° C, 6° C, −10° C, and −52° C in temperature-controlled rooms at the National Research Council Laboratories. The oxygen consumption was converted to heat production from the caloric equivalents for the appropriate R.Q. values. The system for measuring oxygen consumption was identical with that described above except that the slings and stanchions were not used. The animal was pretrained to the face mask and remained absolutely quiet throughout the tests. The measurements were made at the end of each hour of exposure.

Results

Weather Conditions at Mosquito Lake, June 1958

1. Precipitation.—A total of 1.5 in. rain fell between June 2–20 at Mosquito Lake. Snow flurries occurred during the two storms between June 6 and June 10.

2. Sunlight.—Little cloud cover and full sunlight was observed on June 4 and June 12–16. On the remaining days between June 3 and June 11 the sun shone for 0 to 5 hours per day with an average of 1.6 hours/day. It was in this period that most of the tests were carried out.

3. Relative humidity.—Relative humidity varied inversely with temperature but remained between 72 and 100% during the test period. The relative humidity averaged 91% when tests were conducted outside. In the heated building it varied between 50 and 65%.

4. Temperature.—Maximum, minimum, and various mean daily temperatures are shown in Table I. For the most part temperatures ranged between −4° and +1.7° C during the tests. During the period June 2–12 there were only relatively small diurnal variations in temperature, the difference between mean day and night values averaging 1.4° C. After June 12 when the weather warmed up considerably with bright days, diurnal variations in temperature were more marked.

5. Wind velocity.—The average daily wind velocity is given in Table I. In general, winds were light at the foot of the esker near the laboratory building

and exceeded 15 m.p.h. only for short periods of time. The highest wind velocity recorded at Mosquito Lake was 33 m.p.h. on June 9 on top of the esker behind the camp (Table I). In general, wind velocities on the esker were considerably greater than below the esker near the laboratory (Table I). The wind velocity was reduced to about half its value inside the corrals in which

TABLE I

Weather at Mosquito Lake 1958

Date	Temperature °C			Mean wind vel., m.p.h.	Wind chill	
	Maximum	Minimum	Daily mean		Base camp	Top of esker 8 in. above ground
June 2–3	4.4	−3.3	0.6			
3–4	6.1	−3.3	0.5	13.8	1000	
4–5	6.1	2.2	3.3	10.4	700	
5–6	2.2	−1.1	0.6	4.6	750	
6–7	−1.7	−5.5	−4.5	12.8	1070	1150 (9.30 p.m.) (21 m.p.h.)
7–8	−5.0	−6.7	−5.5	14.3	1130	1160 (4.30 p.m.) (22 m.p.h.)
8–9	1.7	−3.3	−2.8	7.6	900	
9–10	0.6	−1.1	0.5	16.3	1000	1150 (4 p.m.) (33 m.p.h.)
10–11	0.6	−1.7	−1.1	7.9	870	1120 (12 a.m.) (24.3 m.p.h.)
11–12	0.6	−1.1	0.5	12.4	940	
12–13	2.8	−1.7	0	4.8	745	
13–14	6.1	0	2.8	2.3	580	
14–15	11.1	2.2	6.7	1.2	445	
15–16	15.0	5.6	10.6	4.6	500	
16–17	18.9	1.1	7.8	7.0	620	

TABLE II

Weather at Beverly Lake, N.W.T., 1959

Date	Temperatures °C			
	Maximum	Minimum	Mean day*	Mean night†
June 2–3	−3.5	−8	−6.0	−3.5
3–4	−1.5	−8	−5.1	−3.5
4–5	−0.5	−7	−2.7	−3.5
5–6	2.8	−3.5	0	−0.5
		(first calves caught)		
6–7	3.0	−1.5	1.2	−0.5
7–8	8.0	−2.5	4.0	0
8–9	7.3	−1.5	3.5	−0.5
9–10	4.5	−5.3	3.2	−2.5
10–11	6.8	−1.5	3.0	1.2
11–12	6.8	−1.7	3.5	−1.5
12–13	8.5	−3.3	4.5	−1.5
		(calving rate highest)		
13–14	5.0	0	3.5	−0.6
14–15	12.0	−2.0	6.8	1.8
15–16	4.0	0	1.8	0.5
16–17	5.6	−0.5	3.5	0
17–18	6.8	−0.5	3.0	0.5

*Day 4.00 hours until 20.00 hours.
†Night 20.00 hours to 4.00 hours.

the calves resided. A similar reduction of wind velocity was found in a corral situated on top of the esker (Table I).

6. Wind chill.—Wind chill has been calculated from the mean temperature and wind velocity values (Table I) according to a formula provided by Siple *et al.* (4). Mean daily wind chill at the foot of the esker varied between 700 and 1130 in the period June 2–11. It was somewhat greater on the top of the esker, but not proportional to the increased wind velocity (Table I) owing to the relatively small increase in the wind chill factor at higher rates of air flow. During the period of bright warm weather June 12–17, the wind chill was relatively low.

7. Weather at Beverly Lake.—The range of daily temperature during the period of calving at Beverly Lake in 1959 is indicated in Table II with means of the warm period (day)* and of the cool period (night).* The small daily fluctuation in temperature was associated with the continuous daylight at that time of the year. Winds were variable from day to day and except for the period June 14–16 were often negligible at "night". Rain associated with high winds occurred for 8 hours on June 15.

Metabolic and Thermal Responses of Newborn Caribou

The baby caribou were highly responsive metabolically to variations in environmental conditions (Table III). When tested in the laboratory under "still" air conditions (0/dry) the heat production was at its lowest for any given temperature. Under these conditions heat production (Y) increased with lowering air temperature (X) (Fig. 1) according to the regression $Y = 14.25 - 0.353X$ (significant at the 1% level) with a correlation coefficient of 0.43. The regression line extrapolates to zero metabolism at a temperature of 43° C.

When the calves were exposed to wind in the 12–21.5 m.p.h. range in addition to cold (12–21.5/dry, Table III) the heat production was significantly higher than when exposed to the same degree of cold without wind (Fig. 1). Finally, the highest heat production was observed when the fur was wet and the calves were exposed to both wind and cold (15–28/wet, Fig. 1, Table III). The respiratory quotient averaged 0.78 in these tests and was not correlated with temperature.

An insulation index, (rectal temp.−air temp.)/(heat production), has been calculated for still air, wind, and wind plus wet conditions (Table III). The insulation averaged approximately 2.9 in still conditions, decreased to 2.0 in windy conditions, and to 1.5 with wind and wet fur. The relative cooling powers of these environmental conditions for caribou were therefore approximately in the ratio of 1:1.5:2.

Body skin temperature and foot temperature fell with decrease in air temperature and with increase of wind or moisture, while rectal temperature was constant under these various conditions (Table III). Skin cooling was also an index of the general metabolic level of the animals for both body skin temperature and foot skin temperature decreased with increased heat production (Fig. 2). There were statistical indications that the different conditions (still air, wind, wind plus wet) did not have the same effect on metabolism and skin cooling, but the physiological significance of these departures are dubious.

*No actual civil twilight existed at this time of year. Average sunset to sunrise is 4 hours at lat. 63° N.

TABLE III

Rectal and skin temperatures, metabolism, and body insulation in relation to environmental conditions

Calf	Body wt., lb	Test conditions (wind, fur, and location)	Air, °C	Rectal, °C	Skin, °C		Heat prod., Cal/hr/ $kg^{3/4}$	Insulation index, °C/Cal
					Body*	Foot		
1	11.7	0/dry in lab	20	40.0	34.4	33.2	4.11	4.86
2	11.3	0/dry in lab	22	40.2	36.8	35.2	9.04	1.99
3	11.7	0/dry in lab	19	39.0	32.5	31.0	6.42	3.11
3	12.8	0/dry in lab	20	40.0	36.6	35.0	9.64	2.07
5	12.0	0/dry in lab	14	39.2	34.4	33.5	12.07	2.07
1	11.6	0/dry/in tent	4	40.5	32.7	29.2	12.23	2.98
2	11.0	0/dry/in tent	4	39.2	34.3	26.5	12.86	2.74
3	11.7	2/dry	0	39.0	31.2	27.7	16.01	2.44
4	11.4	0/dry	−3	39.2	32.4	28.7	15.02	2.81
6	12.0	0/dry	0	39.1	32.7	29.5	17.37	2.25
7	12.5	0/dry	0	38.8	31.8	27.7	12.20	3.18
5	14.5	0/dry	0	38.8	32.2	29.5	14.68	2.64
7	13.4	0/dry	10	39.2	33.7	32.0	8.80	3.32
8	11.4	0/dry	10	38.8	33.3	30.7	6.60	4.36
					Mean insulation 0–2/dry			2.91
1	11.8	15/dry/exposed	−1	38.1	29.3	15.8	19.20	2.04
1	11.6	14/dry/exposed	+3	39.7	31.6	26.0	21.17	1.73
2	11.0	12/dry/exposed	+6	39.0	31.7	22.7	14.20	2.28
3	12.8	14/dry/exposed	−5	41.0	30.7	16.5	21.61	2.13
2	11.6	14/dry/exposed	−5	41.0	26.6	12.5	22.94	2.00
7	19.7	21.5/dry/exposed	+3	39.0	29.7	10.0	20.69	1.74
					Mean insulation 12–21.5/dry			1.99
6	12.0	18/damp/exposed	−1	38.5	28.6	12.5	28.65	1.38
7	10.6	15/damp/exposed	−0.5	39.2	31.5	15.2	20.88	1.90
5	12.0	15/wet/exposed	−1	38.5	30.3	19.5	26.28	1.50
6	13.4	17/wet/exposed	0	39.0	28.6	8.0	31.47	1.24
5	16.1	15/wet/exposed	−0.5	38.8	31.0	10.5	23.51	1.67
7	19.7	28/wet/exposed	+3	38.3			29.69	1.19
					Mean insulation 15–28/wet			1.48

*Body skin mean of three positions on back (neck, shoulder, lumbar) and on side of belly.

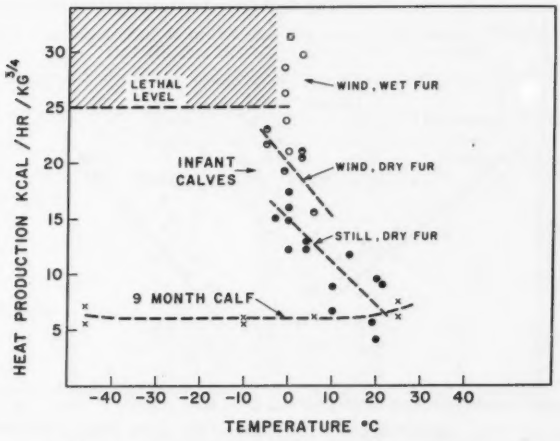

FIG. 1. Heat production as a function of environment temperature in one 9-month calf (X) and infant calves in still air with dry fur (●), wind with dry fur (◓), and wind with wet fur (○). The shaded area and broken line indicates lethal level for prolonged exposure.

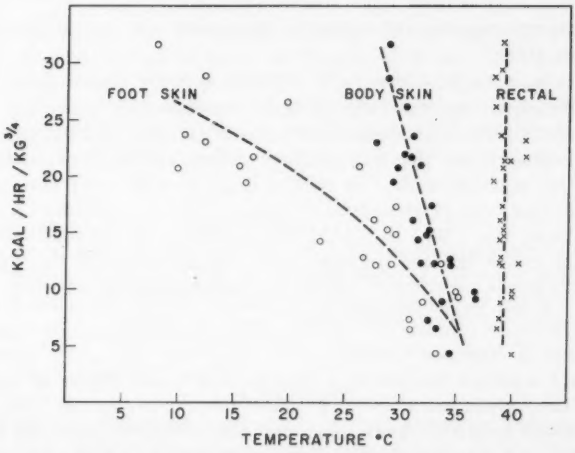

FIG. 2. Heat production as a function of foot temperature (○), body skin temperature (●), and rectal temperature (×) in infant calves.

The oxygen consumption in Fig. 3 was correlated with wind chill values* calculated from the temperatures and wind speeds in Table III. It was found that the heat production (Y) increased with wind chill (X) according to $Y = 4.65 + 0.0164X$ (coefficient of correlation 0.91). The equation was collinear only for "still air" and dry fur, and for wind and dry fur. With wet fur the

*"still air" values taken as 0.2 m.p.h. in these tests owing to turbulence in the cabin.

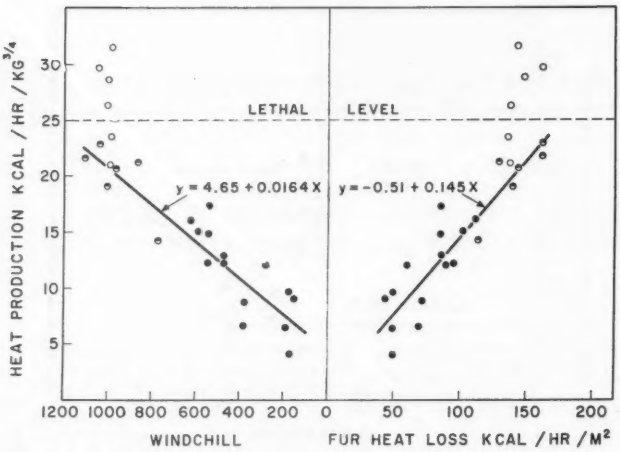

FIG. 3. Heat production as a function of wind chill and heat loss through the fur for infant calves in still air with dry fur (●), wind with dry fur (◓), and wind with wet fur (○). The equations apply for ● and ◓ but not ○, which are significantly higher. The approximate lethal level is indicated by broken line.

metabolic rate was significantly greater than with dry fur at the wind chill value of about 1000.

The metabolic rate has also been correlated with the average heat loss through the fur at various wind velocities (as measured by Lentz and Hart (5)). To calculate heat loss, the conductivity value for the appropriate wind velocity was multiplied by the temperature difference between rectal and air temperature for each animal. The results (Fig. 3) were very similar to those for wind chill. The heat production (Y) increased with heat loss (X) according to the equation $Y = -0.51 + 0.1451X$ (coefficient of correlation 0.93). At zero heat loss the difference between zero metabolism and the extrapolated value (-0.51) is not significant. With wet fur the heat production was again significantly greater than with dry fur.

Cold Resistance of Newborn Caribou

The average weather conditions, exposure times, and degree of hypothermia suffered by the calves are shown in Table IV. At Mosquito Lake calves A, B, and C showed only a slight fall in rectal temperature while calf C showed a fall to 31.5° C in 6.5 hours. In the latter test the wind was stronger and the fur was wetted with driving snow.

In the test on June 8 at Beverly Lake two of the calves suffered little or no hypothermia while the third, which appeared to be sick initially, became hypothermic during the test and later died with a rectal temperature of 32° C, and body skin and foot temperatures of 27° C and 12° C respectively. The newborn calf tested on June 13 was able to maintain normal body temperature for more than 12 hours even though its fur was thoroughly wet with amniotic fluid. Foot temperatures ranged from 28 to 31° C.

TABLE IV

Cold resistance of newborn calves tested under natural conditions at Mosquito Lake, N.W.T., in 1958 and Beverly Lake, N.W.T., in 1959

Date	Calf	Mean temp., °C	Mean wind velocity, m.p.h.	Mean wind-chill	Exposure time, hours	Rectal temp., °C	
						Initial	Final
Mosquito Lake, 1958							
June 6–7	A	−4.5	17	—	22	40.0	37.0
	B	−4.5	17	—	2	40.0	37.0
June 9–10	C	−1.0	23	1150	6.5	38.5	37.0
	D	−1.0	23	1150	6.5	38.5	31.5
Beverly Lake, 1959							
June 8	1	+1.0	12	800	12	38.0	38.0
	3	1.0	12	800	12	38.5	37.0
	4	1.0	16	960	8	38.0	32.0 (died)
June 13	*	+3.0	11	790	14½	39.8	38
June 15	5	+1.5	26 (rain)	1050	5.5	37.5	27.0 (died)
	7	1.5	26	1050	5.5	38.0	36.5 (died)
	8	1.5	26	1050	5.5	38.0	20.0 (died)
	9	1.5	26	1050	5.5	37.5	29.5 (died)

*Post partum calf tested while still wet, a few minutes after birth.

On June 15 four calves were tested under very severe conditions. Heavy rain was falling so that water ran from the animals' fur. All four became hypothermic. Calves Nos. 5 and 8 were dead after 5.5 hours exposure. When released at this time calves Nos. 7 and 9 attempted to find shelter but both became progressively hypothermic and eventually died. Four untested control animals free to find shelter about the camp suffered no adverse effect from the storm.

Thermal Responses of a 9-Month Calf

In contrast to infant calves, the 9-month calf showed no increase in metabolism from 25° C to −55° C (Fig. 1). No evidence of shivering was discerned in any of these tests. At +25° C, however, increase in breathing rate was noticed. Panting was evident during the test at 25° C in which the metabolic rate was 7.5 kcal/hour.

Discussion

Tests of the physiological responses of young calves provide support for the suggestion from field studies that climatic conditions on the barren grounds can be severe enough to lead to mortality should the calves be exposed. In the standing position the calves are very sensitive metabolically to cooling factors of the environment in which the separate effects of cold, wind, and moisture can easily be detected. The calves would be less sensitive in the lying position but as they apparently move long distances under seemingly adverse weather, the test conditions, while possibly more severe, were not unrealistic.

The total increase in heat production of the infant calves was approximately fivefold from the mildest to the most severe test conditions. To evoke such a

response, which is comparable to the maximum seen in deer mice (6) and small mammals in general (7), it was necessary to have windy conditions, particularly in combination with wet fur. During the period of the year when calving occurs, temperatures on the barren lands do not usually fall low enough (1) to evoke such a great response. Storm conditions of winds and precipitation appear to be necessary to produce mortality as observed in this study.

The data presented indicates that the metabolic response of very young animals to field test conditions can be predicted fairly well from meteorological conditions, provided the fur remains dry. The correlation of heat production with wind chill or with calculated heat loss through the fur at various temperatures can be described by single regression lines (Fig. 3). The correlations are tentative since the test conditions were not sufficiently varied to adequately test the assumption of collinearity.

From a theoretical standpoint, use of the animals own fur rather than wind chill (based on cooling of a tin can) should give a better correlation with heat production. On the whole, the data on fur provided a somewhat better correlation with heat production than did wind chill because the former, unlike the latter, can be extrapolated to zero metabolism at zero heat loss. A further difference between the two scales is that wind chill increased only slightly with an increase of wind velocity above 15–20 m.p.h., whereas heat loss through fur increased almost in direct proportion to the increase in wind velocity.

"Thermal wind decrement" or "still shade temperature" (9), another scale of environmental cooling, was not used because values cannot be determined a priori from meteorological conditions. To calculate "still shade temperature" the metabolic rate must be known. A correlation of metabolism with still shade temperature would utilize the same variable as ordinate and abscissa.

It was not possible in this study to define precisely the cold resistance of newborn animals. However, the tests of cold resistance showed that calves could be made hypothermic or be killed if exposed to climatic conditions prevailing during storms on the barren grounds. When wind chill values reach or exceed approximately 1100 or the metabolic level approaches 25 Cal/hour/$kg^{3/4}$ calves would be killed by exposure of long duration. This level of metabolism could therefore be considered the approximate incipient lethal level. This is indicated in Figs. 1 and 3.

Assuming that the data in this study are obtained on a representative sample of calves and are valid within the limits specified, it is feasible to construct a nomogram (Fig. 4) for prediction of the metabolic rate of young caribou from average temperature and wind velocity in the field. The nomogram is based on the correlation between metabolism and heat loss through the fur at various wind velocities and temperatures. Using this nomogram, mortality could be predicted for any combination of conditions leading to a metabolic rate approaching 25. Wet fur reduces the wind velocity and temperature conditions necessary for the lethal effect to be realized, but the heat production cannot be predicted because it depends upon the water content of the fur (5), which is unknown. In the present tests, the increase in metabolism due to wet fur was equivalent to an increase in wind velocity of approximately 10 m.p.h. or to a lowering of temperature by about 10° C.

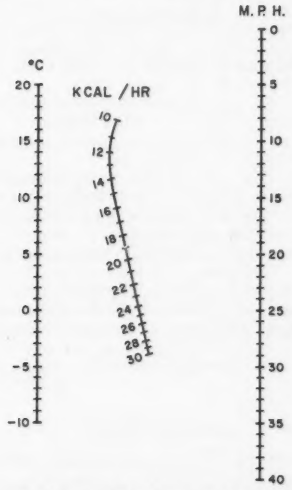

FIG. 4. Nomogram for predicting caribou heat production from mean temperature and wind velocity. Conditions which lead to heat production approaching 25 would be ultimately lethal. Confidence limits for prediction including 95% of values average ± 5.7 cal.

While precipitation tends to make environmental conditions appear more severe than that indicated from temperature and wind alone, this severity is offset by the ability of the calves to seek protection from the cold. In the cold-resistance test of June 15 at Beverly Lake the four animals free to seek shelter suffered no adverse effects during the storm. During an aerial census over the calving area the day after the storm, several more dead calves were seen than on the day before the storm. However, in repeated counts of the number of females with calves made during the calving period the effect of this storm was not evident. Certainly the survival of many species in a seemingly adverse environment can be attributed, at least in part, to their avoidance of the extreme conditions presented.

In contrast to these very young animals, older calves do not rely upon metabolic responses on exposure to cold. Judging from tests on the single 9-month-old calf, the over-all insulation is sufficiently great that they would remain near the thermoneutral state even under severe winter conditions. By their first winter the metabolic responses to cold resemble those of the large arctic mammals studied by Scholander *et al.* (8).

Acknowledgments

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THE RELATION BETWEEN CONCENTRATION OF FOOD AND FEEDING RATE OF DAPHNIA MAGNA STRAUS[1]

F. H. RIGLER

Abstract

Saccharomyces cerevisiae labelled with P^{32} was fed to *Daphnia magna* and feeding rate of *Daphnia* was calculated from uptake of P^{32}. Errors inherent in the method were measured and discussed. An unexpected source of error was due to a rapid uptake of P^{32} during the first few minutes in high concentrations of yeast. An hypothesis is presented to explain this phenomenon.

In concentrations of yeast below 10^5 cells/ml, feeding rate of *Daphnia magna* is proportional to concentration of food. As concentration of yeast is increased above 10^5 cells/ml the feeding rate increases very slightly or not at all. These results are discussed in relation to the supposed inhibition of feeding by high concentrations of log-phase *Chlorella* suggested by Ryther, and Cushing's 'encounter theory' of grazing.

Introduction

Although much interest has been shown in the efficiency of transfer of energy or materials from the primary producers (algae) to primary consumers (zooplankton) in aquatic and marine habitats (1, 11, 14, 17, 18, 20), little serious consideration has recently been given to the relation between feeding rate of zooplankton and concentration of food organisms. The generally accepted view of this relation is based on early work (9, 10, 16) and is clearly stated by Fleming (8), who said, "for any species under a given set of environmental conditions there is apparently a characteristic filtration capacity which is independent of the number of diatoms present in the water". Fleming used 'filtration capacity' as a synonym for 'filtering rate' the term more commonly used to express the volume of water swept clear of food organisms in a given time. Since I will be considering the number of food organisms eaten by a zooplankter, results will be expressed here as 'feeding rate', which means the number of cells consumed by one zooplankter in a given time. Thus feeding rate (cells/animal/hour) equals filtering rate (ml/hour) multiplied by the concentration of food (cells/ml) and to say that filtering rate is constant and independent of the concentration of food is to say that feeding rate is proportional to the concentration of food.

No results inconsistent with the view that the feeding rate of zooplankton is proportional to the abundance of food cells appeared until Ryther (22) published the results of an experiment in which he fed log-phase *Chlorella vulgaris* to *Daphnia magna*. He found that, when *D. magna* were fed in concentrations of *Chlorella* below 2×10^5 cells/ml, feeding rate was, as expected, proportional to concentration of food. But above 2×10^5 cells/ml the rate of increase of feeding rate with food concentration was less than below 2×10^5 cells/ml (Fig. 3). Ryther expected feeding rate of *D. magna* to remain proportional to

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concentration throughout the range of concentration used and concluded that his results differed from those expected because *Chlorella* produces toxins which, in high concentrations, inhibit the feeding of *D. magna*.

Then Marshall and Orr (17) demonstrated that the volume of faeces produced by *Calanus finmarchicus* is proportional to the concentration of *Dunaliella* cells, in which it is feeding, up to 10^5 cells/ml, but above this concentration of algae, production of faeces does not increase. If volume of faeces can be used as a measure of volume of algae ingested, Marshall and Orr's results show that the feeding rate of *Calanus* is proportional to the concentration of the food organism (*Dunaliella*) only up to 1×10^5 cells/ml. Above this concentration the feeding rate of *Calanus* is constant and unaffected by concentration of food.

I believe that Ryther's interpretation of his results is not the only possible one and suggest that his and Marshall and Orr's results indicate a general phenomenon: when a filter-feeding Crustacean encounters low concentrations of food, the feeding rate is limited by the ability of the animal to filter water and hence feeding rate is proportional to concentration of food. But above a critical concentration of food, which will vary with the species of Crustacean and food organism, feeding rate is constant and limited by the ability of the animal to ingest or digest the food cells.

It will be shown below that the feeding rate of *D. magna* may be determined accurately by measuring the radioactivity in the animal after it has been fed radioactive food, when the obvious sources of error are avoided. It will also be shown that when *D. magna* feeds on *Saccharomyces cerevisiae* the relation between concentration of food and feeding rate is the same as that observed by Marshall and Orr for *Calanus finmarchicus* feeding on *Dunaliella*.

Materials and Methods

Daphnia magna were cultured by the method described elsewhere (21). Mature females with a dry weight of 0.23–0.27 mg were used. *Saccharomyces cerevisiae* for feeding experiments was cultured in aerated dextrose medium at 25° C for 16–24 hours in which time a population of 2×10^8 cells/ml developed. One liter of medium contained 5 g Bacto peptone, 2.5 g yeast extract, 0.2 g $CaCl_2.2H_2O$, 0.5 g $MgSO_4.7H_2O$, and 0.5 g $(NH_4)_2SO_4$. It was found that by cutting down the amount of KH_2PO_4 added to this medium below 1 gram per liter, the phosphorus content of yeast could be reduced without reducing the maximum population. The phosphorus content of yeast cells was not affected by increasing KH_2PO_4 content of medium above 1 gram per liter. Low phosphorus yeast was produced by growing a culture to stationary phase in medium without any KH_2PO_4 and high phosphorus yeast in medium containing 1 gram per liter of KH_2PO_4. Unless otherwise specified yeast was grown in medium containing 0.5 g/liter. When radioactive yeast was desired, carrier-free P^{32} was added to the medium after innoculation. When the yeast was used for feeding, the cells contained 95% of the added P^{32}. Before use the cells were centrifuged twice and resuspended in artificial Ottawa river water (A.W.), a solution closely approximating the mineral content of Ottawa river water, but containing no organic compounds (21).

The concentration of yeast suspended in A.W. was determined by counting a sample in a haemocytometer. Usually, 800–1000 cells were counted. Then the desired concentrations to be fed to *Daphnia* were obtained by diluting the stock suspension with Millipore filtered A.W. *Daphnia* were prefed for 1 hour in non-radioactive yeast at the same concentration as the radioactive yeast to which they were to be exposed. The experiment was begun when an equal volume of radioactive yeast suspension was added to the non-radioactive suspension in which they were prefed. The volume of water per *Daphnia* was adjusted so that feeding would reduce concentration of yeast less than 10%. Yeast concentrations presented in the results are averages of the initial and final concentrations.

At the end of the feeding period the animals were removed from the yeast suspension, and rinsed for 5 seconds in filtered A.W. Radioactivity of *Daphnia* was measured as reported earlier (21). Before the radioactivity of yeast was measured the cells were collected on a Millipore filter, washed with A.W., dried on the filter, and covered with a thin plastic film.

The amount of P^{32} in solution during the feeding experiments was very small. In order to measure this P^{32} it was first necessary to concentrate the phosphorus from a large volume of water. This was done by evaporating and digesting 50-ml samples of millipore-filtered water with 0.3 ml of 60% perchloric acid at 180° C for 20 minutes. The digested sample was diluted with distilled water and enough KH_2PO_4 solution was added to give a concentration of 10 μg/ml of phosphorus. The phosphorus was then precipitated as phosphomolybdate and the precipitate collected on a Millipore filter.

Critique of Method of Measuring Feeding Rate

The principle of the method is that radioactive phosphorus is incorporated into the protoplasm of a food organism; *Daphnia magna* are allowed to feed for a given time on this organism, and the radioactivity of the *Daphnia* is then used as a measure of the number of cells eaten. If this method is to give an accurate estimate of feeding rate, several conditions must be met. First, the duration of the feeding experiment must be less than the time for a particle of food to pass through the gut of *Daphnia*. Otherwise, as pointed out by Nauwerck (19) the rate of assimilation of phosphorus, or more precisely, something between assimilation rate and feeding rate will be measured. Second, since the feeding experiment must be short, it is essential that the animals begin to feed at a normal rate immediately they are exposed to the radioactive food. Third, either the food organism must not release radioactive tracer into the water, or *Daphnia magna* must not be able to take up the tracer directly from solution. Finally, it is certain that during the time in which the *Daphnia* are feeding on radioactive yeast, some yeast cells will be digested, a part of the P^{32} they contain will pass into the blood and a part of this P^{32} will be excreted. Therefore it is important to know the magnitude of this loss since it will make the measured feeding rate lower than true feeding rate.

A reliable estimate of the time in which a food particle passes through the gut of *D. magna* has been made by Bourne (1), who found that when this animal is feeding in high concentrations of *Chlorella vulgaris* the average time

is 40 minutes and that as the concentration is reduced the time is longer. However, it seemed desirable to check Bourne's value, since a different food organism, *Saccharomyces cerevisiae*, was used in the present study. This was done with one of the two methods used by Bourne. *D. magna* were fed for varying lengths of time from 5 to 100 minutes on yeast which had been labelled with P^{32}. As indicated by Bourne, if all of the ingested P^{32} is not assimilated, the rate of uptake of tracer will not remain constant but will decrease abruptly at the time when the first radioactive pellets are released. The results of this measurement are presented in Fig. 1, in which radioactivity of *D. magna* is plotted against the time they had fed on radioactive yeast. Figure 1 shows that there is a definite point of inflection in the uptake curve, approximately 45 minutes after feeding on radioactive yeast began. Therefore it appears that *D. magna* passes yeast and *Chlorella* through its intestine at similar rates and that in order to obtain a reliable measure of feeding rate *D. magna* must be fed on radioactive yeast for less than 45 minutes. To obtain measurements reported below a 30-minute feeding time was used.

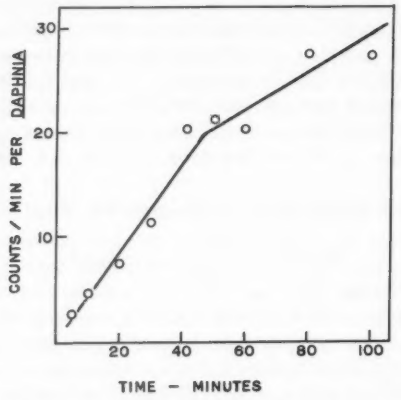

FIG. 1. Uptake of P^{32} by *Daphnia magna* feeding on yeast labelled with 1 count/minute of P^{32} per 1.2×10^4 cells. Yeast concentration was 2.3×10^5 cells/ml.

The second condition which must be met is that the *D. magna* must begin to feed immediately they are in contact with radioactive food. To avoid removing the animals from water the suspension of radioactive yeast was added to the suspension of non-radioactive yeast in which the *Daphnia* had been prefeeding. Nevertheless, violent stirring was necessary to mix the two suspensions quickly and since Ryther (22) reported that agitation of the ambient medium can completely stop the feeding of *D. magna*, measurements of uptake of radioactivity were made during the first 10 minutes of feeding. That feeding was not interrupted is shown by the results of these measurements (Fig. 2). In each case the uptake of radioactivity in the first minute was as rapid as the uptake in succeeding minutes.

Although Fig. 2 conclusively demonstrates that the animals begin to feed on the labelled yeast immediately, it does suggest that another unforeseen source of error exists. There is a strong indication that, when *D. magna* feed in a concentration of 5×10^5 yeast cells per milliliter, uptake of yeast cells is not linear, but is more rapid in the first 4 or 5 minutes than after this time. An hypothesis to explain this phenomenon will be briefly outlined now, although the reasons for proposing it will not be clear until the relationship between feeding rate and food concentration has been presented. It is suggested that *D. magna*, in a yeast concentration of 5×10^5 cells/ml, collects cells more rapidly than it ingests them and that the rate of uptake of P^{32} during the first 4 or 5 minutes of the measurements shows the rate of collection rather than the rate of ingestion of cells. The rate of ingestion is believed to remain constant throughout the experiment and to be shown by the rate of uptake of P^{32} between 4 and 5 minutes and 30 minutes after introduction of radioactive food. Although not shown in Fig. 2, the measurement of uptake of P^{32} by *D. magna* in 5×10^5 cells/ml was continued for 30 minutes, at which time the amount of P^{32} in the *Daphnia* showed that 11.0×10^{-4} cells/animal had been taken up. If the reasoning above is correct, this value is too high and the error can be found by extrapolating to zero time the part of the uptake curve between 4 and 30 minutes. Extrapolation shows that the calculated value is 0.4×10^{-4} cells too high, i.e. the error is 4%.

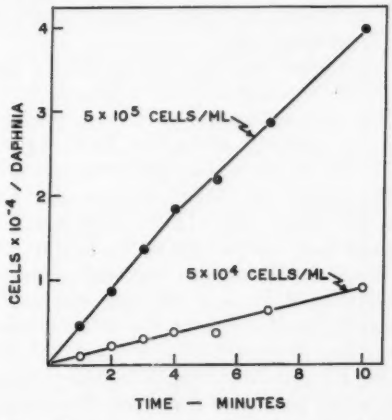

FIG. 2. Uptake of radioactive yeast cells by *Daphnia magna* during the first 10 minutes in radioactive yeast.

The third possible source of error considered was direct uptake of tracer from solution by *D. magna*. In order to reduce this error, yeast was chosen as the food organism and P^{32} as the tracer since it was known that yeast does not exchange phosphorus in a medium containing no glucose (12). Nevertheless, occasional measurements were made of the amount of P^{32} released by yeast into the water used in feeding experiments and the error introduced by direct uptake varied between 0.03 and 0.26%.

One example of the calculation of this error will be given here. In this case 100 ml of water contained 104 counts/minute of P^{32} in solution and 0.3 μg of inorganic phosphorus. Since *D. magna* takes up 1.1×10^{-3} μg of inorganic phosphorus directly from solution in 30 minutes (21) it would take up $(104/0.3) \times 1.1 \times 10^{-3} = .38$ counts/minute of P^{32} in this time. In 30 minutes of feeding the total uptake of P^{32} was 144 counts/minute per animal. Hence the error due to direct uptake of P^{32} was 0.26%.

Finally, the possibility that some of the P^{32} ingested and absorbed by *D. magna* might be excreted during the 30-minute feeding period was investigated. This possible source of error has not yet been accurately evaluated because only one experiment to measure it has been carried out and this experiment was of limited value in that it merely established the upper limit of error. In this experiment, *D. magna* were fed for 30 minutes in a high concentration of yeast cells labelled with P^{32}. At the beginning and end of the feeding period a sample of the yeast suspension was filtered to remove yeast cells and all of the phosphorus in the filtrate was precipitated. Radioactivity of the precipitate was measured. Control samples were prepared from an identical yeast suspension which contained no *D. magna*. Since it seemed likely that rate of excretion of phosphorus would be related to the phosphorus concentration in the food organism, one group of animals were fed on yeast with a low phosphorus content and another group on yeast with a high phosphorus content. As usual, feeding rate was calculated from measurements of radioactivity of yeast and radioactivity of the animals after 30 minutes of feeding on the yeast (Table I, column 1–3).

The results (Table I, column 4) show that in control suspensions there was a negligible increase of P^{32} in solution in 30 minutes, but in suspensions containing *D. magna*, although the amount of P^{32} was initially the same as that in the controls, it increased markedly during the 30 minutes. If it is assumed that all of this P^{32} was excreted by *D. magna* then this amount should be added to that remaining in the animals at 30 minutes to give the total P^{32} ingested. The percentage loss of P^{32} is shown in Table I, column 5. Although the feeding rates of the two groups were similar, those feeding on yeast containing 0.43 μg $P/10^6$ cells excreted 3.6% of the ingested phosphorus whereas those feeding on yeast containing 0.08 μg $P/10^6$ cells lost only 2.1%. These values merely show the upper limit of the error due to excretion of P^{32} because some of the P^{32} might have been released by the yeast. Although the control suspensions released no P^{32}, possibly the *Daphnia* excreted enough organic metabolites into the water to enable the yeast to exchange a small part of its phosphorus with phosphorus in solution. Since the specific activity of phosphorus in the yeast was more than 10 times greater than that of phosphorus in solution, this would have caused an increase of dissolved P^{32}.

The Relation Between Concentration of Food and Feeding Rate

Feeding rates of *D. magna* were measured in A. W. containing concentrations of yeast from 9×10^3 to 1×10^6 cells/ml. Fifty *Daphnia* were used at each concentration. The results (Fig. 3) show that in concentrations below 10^5 cells/ml the feeding rate of *D. magna* is proportional to concentration of

TABLE I

Measurements of increase of P^{32} in solution caused by *Daphnia magna* feeding on yeast containing different amounts of phosphorus and labelled with P^{32}

	(1)	(2)	(3)	(4)				(5)
				P^{32} in solution (counts/min/ml)				
				Yeast		Yeast + *Daphnia*		
	Yeast concn. (cells/ml)	P^{32} in yeast (counts/min/ml)	P^{32} per *Daphnia** at 30 min (counts/min)	0 min	30 min	0 min	30 min	Error† (%)
Low P yeast (0.08 μg P/10^6 cells)	1.60×10^5	455	529	0.28	0.32	0.29	2.61	2.1
High P yeast (0.143 μg P/10^6 cells)	1.34×10^5	124	148	0.53	0.54	0.51	1.58	3.6

*In the suspension of low-phosphorus yeast there was one *Daphnia* per 5.07 ml and in high-phosphorus yeast, one *Daphnia* per 5.2 ml.
†The error is the amount by which the calculated feeding rate is lower than the true feeding rate if the increase of P^{32} in solution is due to excretion of P^{32} by *Daphnia*.

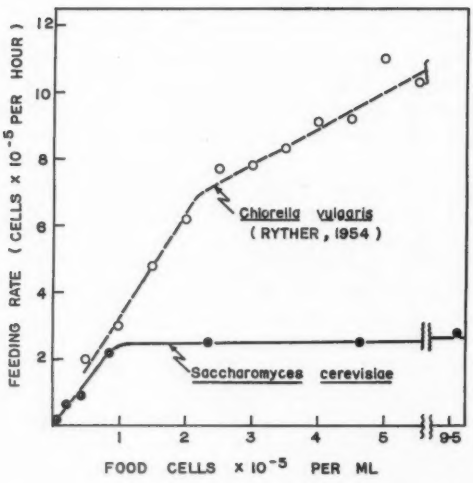

FIG. 3. The feeding rate of *Daphnia magna* in different concentrations of yeast calculated from uptake of P^{32} and in *Chlorella vulgaris* (from Ryther, Table I, p. 528) calculated from decrease of algal cells in the ambient medium.

food, i.e. filtering rate is constant from 0 to 10^5 cells/ml at 2.6 ml/hour. But above 10^5 cells/ml a 10-fold increase in the concentration of yeast cells has almost no effect on feeding rate. Hence, it appears that below 10^5 yeast cells/ml the feeding rate is limited by the amount of water the animal can filter, but above 10^5 cells/ml it is limited by some other factor.

Discussion

Conover *et al.* (4) were reported by Corner (5) to have found that the feeding rate of *Calanus finmarchicus* measured by feeding it radioactive food is only 1/20th of the rate obtained by the traditional method of measuring decrease of algal cells in suspension. This discrepancy should not be interpreted as indicating that the use of radioactive food is an unsatisfactory method of measuring feeding rate. I have shown that the errors inherent in the method are small and can be largely eliminated. The largest errors were due to excretion of ingested tracer and a rapid initial uptake of radioactive food, but neither was greater than 4%. Since one is positive and the other negative they tend to cancel each other. If necessary, both errors could be reduced or measured accurately.

Loss of P^{32} could be reduced by feeding yeast containing less phosphorus. The results in Fig. 3 were obtained by feeding *D. magna* on yeast containing 0.3 μg P/10^6 cells. Hence the results are probably 3% low (Table I). This error could be reduced to 2% by using yeast containing 0.08 μg P/10^6 cells and probably it could be reduced further by shortening the feeding experiment, thus allowing less absorption and excretion of P^{32}.

The existence of the second error was suggested by Fig. 2, which shows a more rapid uptake of P^{32} in the first 4–5 minutes by *D. magna* feeding in a concentration of 5×10^5 cells/ml but not in a concentration of 5×10^4 cells/ml. A similar observation was made by Bourne (1), who measured the uptake of C^{14} by *D. magna* feeding on C^{14}-labelled *Chlorella vulgaris*. The cause of this phenomenon is not known, but it can, perhaps, be deduced from consideration of the relation between feeding rate and concentration of food, and of the feeding mechanism of *D. magna*. When less than 10^5 cells/ml of yeast are available the feeding rate is proportional to concentration of food. Apparently the animal is filtering water at a maximum rate and ingesting all cells collected. But increasing the concentration above 10^5 cells/ml does not increase the rate of ingestion. Therefore *D. magna* must be able to limit its intake of food, either by reducing the rate at which it pumps water over the filtering setae or by pumping intermittently, or by rejecting some of the collected cells. There is no direct evidence showing which of these mechanisms operate, but it is known that *Cladocera* can eject particles from the food groove. Food particles are filtered from the water on the endites of the third and fourth thoracic appendages, immediately swept into the food grooves by combs on the second and third appendages, and are carried forward to the anterior end of the groove (3). Here the food accumulates until the maxillules and mandibles pass it into the oral cavity. Several workers (2, 7, 13, 15) have observed that if particles too large to be eaten reach the food groove they are ejected from the groove by the abreptor. Possibly this mechanism also operates in dense suspensions of food and removes excess food from the groove.

Although any of the above mechanisms might prevent feeding rate from increasing as the concentration of yeast is increased above 10^5 cells/ml, the rapid initial uptake can be easily explained if the last mechanism operates. Consider an animal feeding in any concentration of non-radioactive yeast above 10^5 cells/ml. Let us say it is collecting cells at a rate of 15 units/3 minutes and ingesting cells at a rate of 10 units/3 minutes. To keep the number of cells in the food groove constant it must reject cells at a rate of 5 units/3 minutes. When radioactive yeast is added to the non-radioactive, both the food groove and gut will contain only non-radioactive yeast (Fig. 4), and the animal will begin to collect radioactive yeast at 15 units/3 minutes. After 3 minutes the food groove contains 15 units of radioactive yeast and, of the non-radioactive which was in the groove, 10 units have passed into the gut and 5 have been rejected (Fig. 4). Thus the uptake of radioactivity in the first 3 minutes gives a measure of rate of replacement of food in the food groove. However, in subsequent 3-minute intervals, rejected food consists entirely of radioactive yeast and uptake of radioactivity will be 10 units/3 minutes, i.e. it will show rate of ingestion of yeast.

In the example above, 'radioactive yeast' means that half of the cells contain P^{32} and when the food groove was said to contain only radioactive yeast, it contained a group of yeast cells of the same specific activity as those in suspension in the ambient water. The argument was simplified by assuming that only non-radioactive cells are rejected during the first 3 minutes of the measurement. This assumption is probably wrong, but even if rejection were completely random the rate of uptake of radioactivity by *Daphnia* would

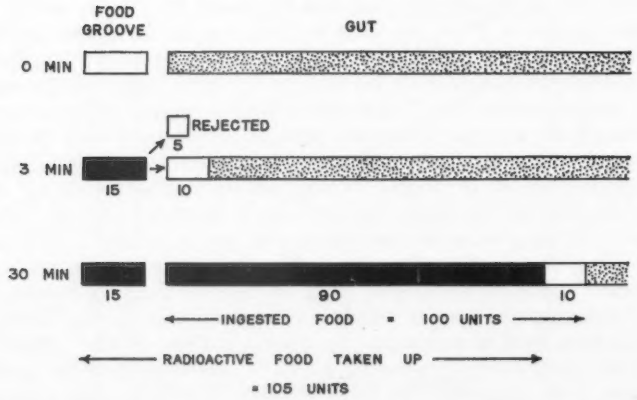

FIG. 4. Diagrams showing hypothetical amounts of non-radioactive and radioactive food in the food groove and gut of *Daphnia magna* in yeast concentrations higher than 10^5 cells/ml when radioactive yeast is added to the water, and 3 and 30 minutes after addition of radioactive yeast. The ratios of amount of food in the food groove to the amount in the gut and of ingested to rejected food were chosen arbitrarily.

The length of horizontal bars is proportional to the volume of food. The stippled area represents non-radioactive yeast in the gut, and the blank area, the amount in the food groove when radioactive yeast is added to the ambient medium (0 minute). The black area represents radioactive yeast.

still be more rapid until the food groove contained only radioactive cells. However, if the turnover time of yeast in the food groove were 3 minutes, as in the example, then the initial rapid uptake would last longer than 3 minutes and the initial rate of uptake of radioactivity would not give an accurate measure of rate of collection of cells.

It is believed that in concentrations of yeast below 10^5 cells/ml the rate of collection of cells is less than the maximum possible ingestion rate. Hence no rejection takes place and the rate of uptake of P^{32} by *Daphnia* remains constant.

The same example shows the source of the error introduced by the tracer method of measuring feeding rate (bottom bar, Fig. 4). During 30 minutes in radioactive yeast, *Daphnia* ingests 100 units of food, 90 units radioactive food and 10 units of non-radioactive food. But 105 units of radioactive food is taken up in this time and hence feeding rate calculated from uptake of P^{32} in 30 minutes will be 5% too high.

If this hypothesis is correct the error can be eliminated by calculating feeding rate from the difference between P^{32} in the animals at 5 minutes and 30 minutes after immersion in radioactive yeast at concentrations above 10^5 cells/ml. Below this concentration, the present method appears to be accurate.

Three techniques have now been used to show the relation between concentration of food and the feeding rate of planktonic Crustacea. Ryther (22) measured the decrease in algal cells in the ambient medium caused by the

feeding of *D. magna*; Marshall and Orr (17) measured size and rate of production of faecal pellets, by *Calanus finmarchicus* in different concentrations of algae; and I have measured the uptake of P^{32} by *D. magna* feeding on yeast labelled with P^{32}. Marshall and Orr's results and mine suggest that above a critical concentration of food, the feeding rate is independent of concentration of food. I believe this will prove to be a general phenomenon. However, Ryther's results, although they show feeding rate to be proportional to algal numbers below 2×10^5 cells/ml, suggest that feeding rate continues to increase, but less rapidly, as algal concentration is increased above this point. Ryther, in interpreting this observation, felt that feeding rate should be proportional to food concentration throughout the range of concentrations he used and concluded that in concentrations above 2×10^5 cells/ml it is depressed by the toxic effects of chlorellin, an antibiotic produced by *Chlorella*. I would suggest that this observation need not be considered as evidence that *Daphnia* is poisoned by *Chlorella* and further, that the interesting feature of Ryther's results is that the feeding rate, rather than remaining constant above the critical concentration of *Chlorella*, continues to increase. If Ryther's results are correct, the simple relation I have proposed, on the basis of Marshall and Orr's and my observations, is not general. Obviously, more accurate measurements of the feeding rate of zooplankton on different food organisms must be obtained before this problem can be settled.

Finally, the applicability of Cushing's (6) 'encounter theory' of grazing to the feeding of *D. magna* should be considered. Cushing developed a theoretical relationship between the concentration of diatoms and the filtering rate of marine copepods. (Note that *filtering rate* is considered in the ensuing discussion.) He assumed that the maximum volume of water filtered is equal to the area of the collecting appendages times the distance travelled by the animal and that when the animal encounters a food particle it stops filtering for a finite time to eat the particle. Thus the maximum volume is filtered when no particles are encountered. As the concentration of food is increased the amount of time spent eating increases and the amount sweeping decreases. Thus filtering rate decreases continuously as the concentration of food increases, according to the formula $y = a/(1 + bx)$, in which y is the filtering rate, x is the concentration of algal cells, and a and b are constants when a given organism is feeding on cells of uniform size.

Although the formula might adequately define the filtering rate of copepods feeding on large diatoms, I do not agree with Cushing that it can be applied to *D. magna* feeding on small algal cells. First, *D. magna* collects and ingests food particles simultaneously. It never stops collecting to ingest a cell. Second, the experimental results presented above do not conform with Cushing's equation: they show that over a wide range of concentrations filtering rate does remain constant.

Acknowledgments

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INFLUENCE OF TEMPERATURE AND HUMIDITY ON LONGEVITY, FECUNDITY, AND RATE OF INCREASE OF THE MITE TYROPHAGUS PUTRESCENTIAE (SCHRANK) (ACARINA: ACARIDAE) REARED ON MOLD CULTURES[1]

I. RIVARD

Abstract

Adult females of *Tyrophagus putrescentiae* (Schrank), a mite that infests stored food products, were reared individually on mold cultures at all combinations of five temperatures and four humidities. A definite diminution in life usually occurred with increase in either temperature or humidity, and the oviposition period was the most affected. Total egg-production and rate of laying were highest at 72.5° F and 90% R.H., and both generally decreased with an increase in temperature or decrease in humidity from these values. Peak of egg-laying, however, was reached sooner at higher temperatures but egg-production was maintained at relatively high levels for shorter periods. Rate of increase was greatest and approximately the same at 90% R.H. within the range 72.5° to 81.5° F.

Introduction

The effects of various combinations of temperature and humidity on the mortality and rate of development of the immature stages of *Tyrophagus putrescentiae* (Schrank), a mite that infests stored food products, were recently described (3). Mortality was lowest at 72.5° F and 80% to 90% R.H. and development was generally accelerated by an increase in either temperature or humidity. Data on the influence of these two factors on survival and the time required to complete one generation, however, are not sufficient to determine the limits of these physical conditions that are most favorable for population increase. The latter depends largely on a high rate of reproduction, which in turn is regulated by the longevity and fecundity of the adults. This is a report of further experiments designed to investigate that aspect, and information gathered from both studies is used in this paper to calculate the innate capacity of the species for increase at these combinations of temperature and humidity.

Materials and Methods

The mites used were from stock cultures bred for several generations on a mold, *Aspergillus* sp. They were reared at constant humidities of 70%, 80%, 90%, and 100% R.H. and at constant temperatures of 68.0°, 72.5°, 81.5°, and 86.0° F as in the experiments described earlier (3). The longevity and fecundity of 15 *T. putrescentiae* females were studied individually in each series of tests in the same manner as in the ones conducted at 77.0° F (2).

The numbers of eggs laid and the durations of the various periods of adult life of the females were recorded and the average values calculated to the

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first decimal. Data for rearings at 77.0° F were already available from previous experiments (2). The "*t*" test was used to determine significance at the 5% level.

Results and Discussion

Longevity

An increase in either temperature or humidity usually shortened the average lengths of various periods of female adult life, though there was much variation between the individuals at any given set of conditions (Table I).

The preoviposition period lasted at least 1 day and rarely exceeded 4 days. It was usually shortened by an increase in temperature, but it was significantly longer only at 68.0° F for all the humidities tested, except at 70% R.H. when a rise of approximately 13 degrees was necessary to shorten that period significantly. On the other hand, there was little influence from a change in humidity and the mites started to lay their eggs sooner with an increase in humidity only at 72.5° F.

Similarly the oviposition period was generally shorter at higher temperatures, but except at 90% R.H. an increase from 68.0° to 77.0° F did not shorten that period significantly. In relation to humidity it was shortest at 100% R.H. in the range 68.0° to 77.0° F; above 77.0° F the trend was reversed when drier conditions apparently favored deposition of eggs over a longer period. The oviposition period was longest at 72.5° F and 80% R.H. and shortest at 86.0° and 70% R.H., whereas at 68.0° F the mites laid their eggs in approximately the same time at all humidities. There was also no significant difference between 70% and 80% R.H. or 90% and 100% R.H., and between 70% and 100% R.H. or 80% and 90% R.H. at 77.0° and 81.5° F respectively.

The postoviposition period was relatively longer at 68° and 72.5° F than at the higher temperatures. The humidity had little effect on the length of that period in the temperature range 77.0° to 86.0° F, but at 68.0° and 72.5° F the females died sooner at 70% and 80% R.H. respectively.

The total adult life of *T. putrescentiae* was significantly longest at 68.0° F and 90% R.H., and shortest at 86.0° and 70% R.H. Longevity also generally decreased with an increase in temperature above 72.5° F, but at the same time was little affected by a change in humidity except at 77.0° F when it was significantly greater at 70% and 80% R.H. than at 90% or 100% R.H. The percentage of survivors decreased much more rapidly at the higher temperatures, especially at 90% R.H. or lower (Fig. 1). For each humidity level the longevity curves at 68.0° and 72.5° F were strikingly similar, and so were those at 81.5° and 86.0° F. That at 77.0° F was intermediate, but with decreasing humidity tended to resemble those at the higher temperatures.

Fecundity

The highest average number of eggs per female was laid at 72.5° F and 90% R.H., and egg production decreased at higher or lower temperatures (Table II). Approximately the same quantity of eggs was deposited at 68.0° and 77.0° F for any given humidity except 70% R.H. At the temperatures below 86.0° F egg production was always greatest at 90% R.H., though there was no significant difference between the numbers deposited at 80% and 90% R.H. A

TABLE I

Duration in days of life of 15 adult females of *T. putrescentiae* at various combinations of temperature and humidity

Temp., °F	Relative humidity, %	Preoviposition period		Oviposition period		Postoviposition period		Total adult life	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
68.0	70	2–5	3.0	26–53	35.2	1–13	4.2	32–66	42.4
	80	2–5	2.9	18–51	37.5	1–42	12.5	28–78	52.9
	90	2–9	3.3	14–45	33.3	4–42	24.2	33–83	60.8
	100	2–6	3.1	12–44	29.1	0–45	12.0	25–63	44.2
72.5	70	2–4	2.7	13–67	31.8	1–47	16.7	29–71	51.2
	80	2–4	2.4	22–62	42.2	1–43	8.0	26–76	52.6
	90	1–2	1.9	17–55	33.0	3–47	20.4	25–76	55.3
	100	1–2	1.9	17–41	30.1	1–31	13.4	26–63	45.4
77.0	70	1–4	2.5	15–63	36.7	1–9	3.8	20–70	43.0
	80	1–4	2.1	15–49	32.9	1–7	2.7	17–58	37.7
	90	1–3	1.9	7–40	24.0	1–8	2.7	12–50	28.6
	100	1–4	2.1	7–43	23.2	0–10	2.5	11–47	27.8
81.5	70	2–3	2.3	6–18	12.0	1–12	3.2	9–26	17.5
	80	1–6	2.4	6–27	15.5	0–14	4.2	12–35	22.1
	90	1–4	1.9	12–24	16.8	0–7	3.1	16–33	21.8
	100	1–3	2.0	6–26	12.9	1–14	2.8	9–31	17.7
86.0	70	1–3	1.9	2–13	6.4	1–7	2.6	6–18	10.9
	80	1–5	2.7	3–20	9.9	0–10	3.3	7–26	15.9
	90	1–4	2.3	5–16	9.9	0–15	3.4	10–21	15.6
	100	1–4	1.9	5–18	10.8	0–16	6.3	7–27	19.0

further increase to 100% R.H. usually caused a significant drop in the fecundity, but at 86.0° F the highest humidity was most favorable to the egg production.

The highest average number of eggs per female per day was also laid at 72.5° F and 90% R.H. Because of a substantial reduction in the length of the oviposition period with an increase in temperature, however, a rise to 86.0° F was necessary to lower that rate significantly at each humidity level. On the

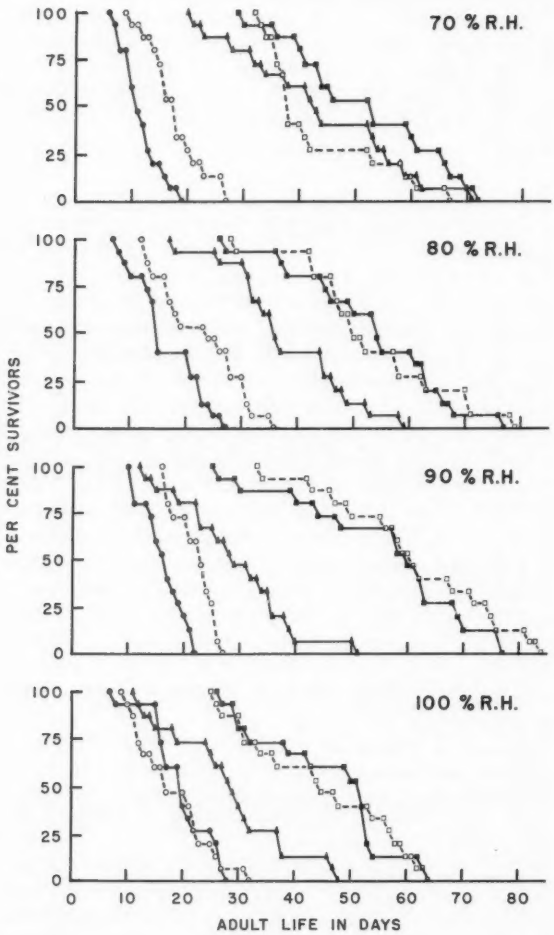

FIG. 1. Longevity of adult *T. putrescentiae* females at various combinations of temperature and humidity. (Closed squares, 68.0° F; open squares, 72.5° F; closed triangles, 77.0° F; open circles, 81.5° F; closed circles, 86.0° F).

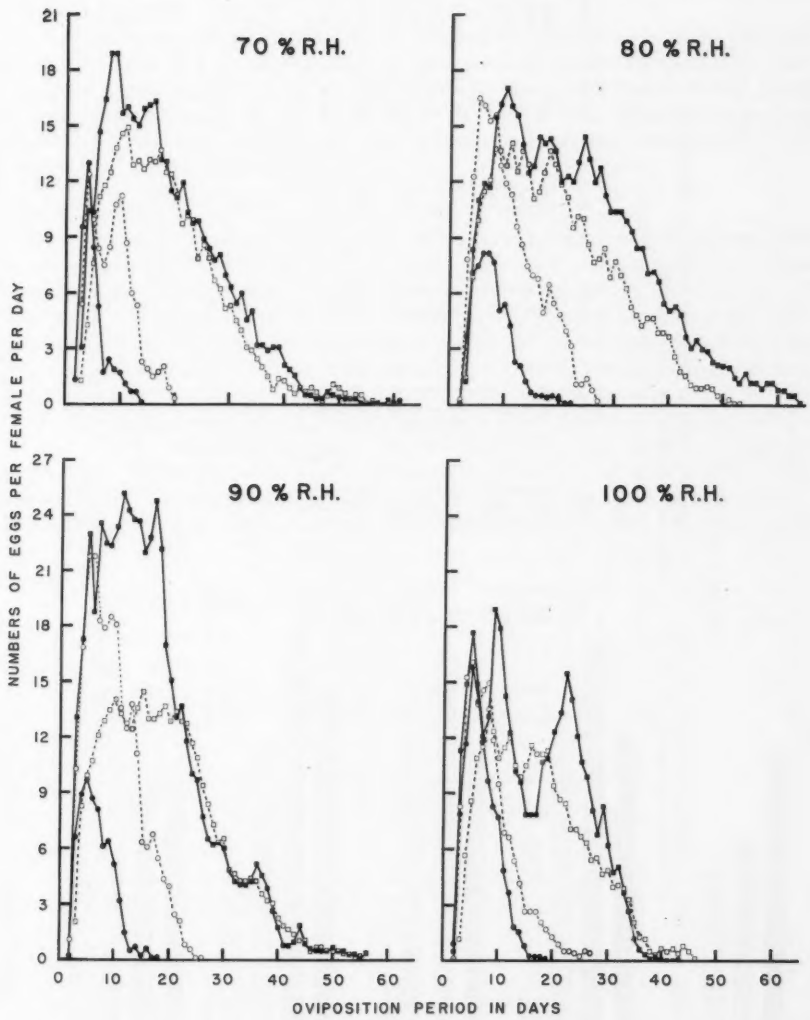

FIG. 2. Age – specific fecundity of adult *T. putrescentiae* females at various combinations of temperature and humidity. (Closed squares, 68.0° F; open squares, 72.5° F; open circles, 81.5° F; closed circles, 86.0° F).

TABLE II

Average numbers of eggs laid per week per female alive at the beginning of the experiment for 15 females of *T. putrescentiae* at various combinations of temperature and humidity

Temp., °F	Relative humidity, %	Week									Total
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	
68.0	70	62.5	94.5	79.7	47.8	20.5	6.6	4.9	1.6	—	318.1
	80	69.5	88.9	81.8	57.5	37.1	18.6	4.6	0.1	—	358.1
	90	69.5	93.9	91.9	60.6	30.5	14.7	4.6	1.0	—	366.7
	100	63.9	76.8	67.3	40.7	19.9	3.8	0.6	—	—	273.0
72.5	70	92.7	110.4	80.8	56.3	30.4	13.3	2.9	1.0	0.5	388.3
	80	77.0	102.5	91.6	84.7	60.9	34.2	18.1	8.9	4.0	481.9
	90	118.4	164.9	128.8	58.2	32.9	15.7	5.7	2.1	—	526.7
	100	84.6	91.2	78.7	70.1	24.3	0.8	—	—	—	349.7
77.0	70	36.8	28.3	26.9	18.7	15.0	8.1	4.6	1.6	0.7	140.7
	80	83.0	76.4	65.3	40.8	19.1	7.0	1.9	—	—	293.5
	90	112.4	96.5	57.0	42.5	13.1	0.5	—	—	—	322.0
	100	111.9	82.4	37.5	13.1	7.6	2.8	—	—	—	255.3
81.5	70	62.7	36.9	4.9	—	—	—	—	—	—	104.5
	80	84.1	68.9	35.9	4.6	—	—	—	—	—	193.5
	90	108.0	93.7	31.6	1.6	—	—	—	—	—	234.9
	100	83.3	47.2	11.8	2.0	—	—	—	—	—	144.3
86.0	70	41.6	6.3	—	—	—	—	—	—	—	47.9
	80	42.5	21.1	2.3	—	—	—	—	—	—	65.9
	90	48.5	18.7	0.8	—	—	—	—	—	—	68.0
	100	71.9	28.7	0.7	—	—	—	—	—	—	101.3

other hand, the rate of egg-laying usually increased with increase in humidity up to 90% R.H., except at 86.0° F when maximum rate was obtained with 100% R.H.

On the average the peak was reached during the second week of oviposition at 68.0° F and 72.5° F, and during the first week at higher temperatures, but egg-laying was maintained at a relatively high rate much longer at the lower temperatures (Fig. 2). In the range 68.0° to 77.0° F the mites laid a greater portion of their eggs much sooner with increasing humidity, whereas at 81.5° and 86.0° F the reverse was true.

Rate of Increase

As in the experiment conducted at 77.0° F (2), Howe's method (1) was used to determine the rate of natural increase of *T. putrescentiae* at the various combinations of temperature and humidity. Mortality during development of the mites and mean developmental period of the females were reported previously (3). Sex ratio of the adults was also found to be approximately 1:1. The oviposition period was divided into units of 1 week, and from Fig. 2 it was possible to calculate the total developmental period of the females to the middle of the first week of egg-laying. The various weekly oviposition rates per female (Table II) were corrected for sex ratio and mortality, and new sets of figures were obtained that represented the effective numbers of eggs laid. Then, on the assumption that eggs were deposited only during the first week of oviposition, a minimum value of r (intrinsic rate of increase) was estimated. The equivalent

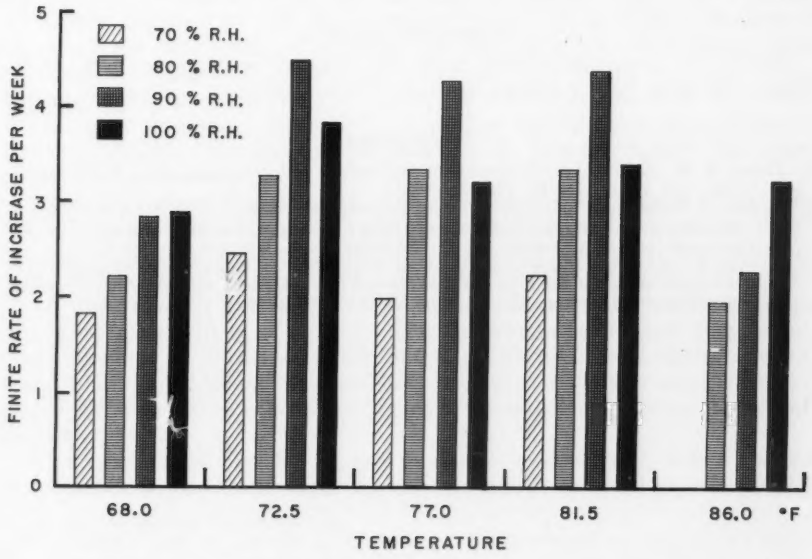

FIG. 3. Rate of increase of *T. putrescentiae* mites at various combinations of temperature and humidity.

egg number in the first week was calculated according to the appropriate table of weighting factors provided by Howe (1). Finally the approximate value of r was estimated and the corresponding value of λ (finite rate of increase) determined (Fig. 3).

Greatest potential of population increase was found to occur at a humidity of 90% R.H. and a temperature of 72.5° to 81.5° F, when the mites could multiply about four and a half times per week. When the humidity was 10% lower or higher in that temperature range the rate of increase dropped to approximately three and a half times per week. A further decrease to 70% R.H. caused the mites only to double the population within the same period. At the lowest temperature the mites multiplied more rapidly with increased humidity from 70% to 90% R.H. Even at small numbers of eggs were laid at 86.0° F and 70% R.H., populations of *T. putrescentiae* could not increase because, under these conditions, no mites would develop to the adult stage (3). At that high temperature the humidity had to be increased to 100% R.H. to obtain maximum population growth. It is apparent from the above results that *T. putrescentiae*, when reared on mold cultures, would multiply most rapidly if the temperature is maintained somewhere in the range 72.5° to 81.5° F and the humidity kept at about 90% R.H. These rates are mostly influenced by the direct effects of these two environmental factors, though humidity might also affect mold growth to some extent (3). If other foods, however, such as flours or cereal flakes, were used, the rates of increase would probably be much less (T. Burnett, personal communication) and would likely vary in a somewhat different manner in relation to the temperature and humidity.

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OBSERVATIONS ON EXPERIMENTAL INFECTIONS WITH ECHINOCOCCUS IN RODENTS[1]

GLORIA A. WEBSTER AND T. W. M. CAMERON

Abstract

Experimental infections in rodents with *Echinococcus multilocularis sibiricensis*, *Echinococcus granulosus canadensis*, and *Echinococcus granulosus* (Lebanon origin) both by ingestion of onchospheres and by parenteral injection are recorded. The microscopic anatomy of the larval parasite and of the host reaction are discussed and illustrated in detail. Voles and cotton rats display little resistance to the development of the cyst of *E. m. sibiricensis* and ultimately destroy the host, while collared lemmings and golden hamsters overcome the cyst and destroy it. Muskrats and certain white mice are intermediate in their effects. In all cases, however, some degree of degeneration of the parasite into the alveolar form is shown. White rats, guinea pigs, and rabbits could not be infected with this parasite. *E. g. canadensis* was developing in the lung of one AKR strain mouse while in the chinchilla the parasite commenced to develop in the lung but was overcome at an early stage. No development took place in the other species of hosts. *E. granulosus* (Lebanon) appeared to be developing successfully in some cotton rats and white mice fed on onchospheres.

Introduction

With the advent of the work of Rausch and Schiller (15) and Vogel (20) on the life history of *Echinococcus multilocularis*, the rodent became an important laboratory tool for studying various aspects of echinococcosis. For the first time, it was possible to maintain hydatid infections which grew rapidly and luxuriantly in animals which could be kept conveniently in the laboratory. Sadun *et al.* (18) found that cotton rats were excellent laboratory hosts; all the rats which he infected experimentally with *E. multilocularis* eggs produced massive cysts. As our findings were similar we used these animals routinely as a source of material for in vitro studies until they were found to be extremely susceptible to murine salmonellosis. This made it necessary to find another host as receptive to hydatid disease but more resistant to *Salmonella*.

Although various investigators have infected a wide variety of rodents with *E. multilocularis* eggs, their reports present conflicting evidence about the susceptibility of a given species. Vogel (20) and Mankau (12) established infections in white mice, while Rausch and Schiller (17) and Sadun (18) found these animals were not susceptible. The inconsistency of these reports prompted Yamashita *et al.* (25) to infect various strains of mice. They found that strain was an important factor, and that infection ratios varied between 8 and 100%. In addition, these authors reported that the host-tissue reaction and morphological aspects of the hydatid represents two distinct types of development.

A range of receptivity also appears to exist in wild rodents; certain species of voles (15, 17) and wild mice (15, 18) are readily infected experimentally

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while others show a strong resistance. Lubinsky (10) was able to establish primary infections in only 50% of his gerbils while Yamashita (25) obtained a 100% infection rate. The hamster, by all accounts, is not a good host; development is abortive and the cysts usually degenerate (10, 25). It is also generally agreed that white rats, guinea pigs, and rabbits are not susceptible to infections with *E. multilocularis* eggs.

Voluminous cysts are produced rapidly when *E. multilocularis* scoleces are inoculated intraperitoneally into cotton rats. This secondary type of development also occurs in gerbils, but Lubinsky (11) states that in this host the growth rate is considerably slower and the volume of cyst material approximately one-third of that obtained in cotton rats. Lubinsky also obtained successful serial passages in both the gerbil and the cotton rat. Data on secondary infection in mice are conflicting. Yamashita (24) found that in mice of the *dd* strain, scoleces died at an early stage of vesiculation. In contrast, Lubinsky (11) obtained good cyst growths which were usually fertile and which he carried through three subsequent serial transfers.

Our supply of *Echinococcus granulosus* material from northwestern Canada is seasonal and available only during the annual elk and reindeer kills. Both primary and secondary infections in rodents were made in an attempt to ascertain whether there was any possibility of maintaining this species in laboratory animals.

There are several records in the literature of attempts to establish primary infection of *E. granulosus* in rodents. Negative results were obtained by Rausch and Schiller (15) and Hutchison and Bryan (9). Dévé (2, 3, 4) infected numerous rodents with segments of "ténia echinocoque" and recovered hydatid cysts from the kidney, pleural cavity, and lungs of a rabbit; the pleural cavity of a mouse and squirrel; and the spleen and pleural cavity of a cat. In one instance, the cyst was fertile. Yamashita (23) successfully infected two of 20 *dd* strain mice. In the first case, three univesicular hydatid cysts were present in the liver, while the second mouse had a single cyst of 10-mm diameter in the right pleural cavity.

Interesting comparisons on secondary *E. granulosus* infections in rodents are given by de Waele and de Cooman (21). Using scoleces of horse origin they infected successfully white mice and white rats; rabbits were negative. Dévé (7) found that, with material of sheep origin, white mice and rabbits were receptive, rats refractory. Due to this difference, de Waele and de Cooman proposed that scoleces originating from the hydatids of different hosts, although morphologically indistinguishable, were physiologically different and, therefore, specific. Schwabe (19) reported that with material from a human hydatid, secondary *E. granulosus* cysts developed in young white mice.

Histopathology

The environment of the hydatid cyst is the host in which it occurs and its development is influenced by the reaction of this environment to its presence. This differs whether the parasite enters the host as an onchosphere and, therefore, passes through the digestive system, the portal system, and the liver

or whether it enters the body parenterally and so bypasses the digestive system. The first method is the natural route of entry while the second is artificial. In the natural infection with onchospheres, the acellular larva, when it reaches the liver, presumably through a blood vessel, is surrounded, within a very few hours, by epitheloid cells and leucocytes, including some eosinophiles. There is a toxic necrosis of these cells which causes the formation of syncytial giant cells and continues until a hyaline membrane is laid down. The intensity of this reaction probably determines whether the parasite can continue its development or be overcome by the host's reaction and be killed. If the reaction is relatively slight, either in the toxicity or quantity of fluid produced and passed through the developing germinal layer into the host tissue, the parasite continues to grow, and as it grows, it lays down the first layer of the cuticular hyaline capsule. This apparently limits the excretion of some of the previous components of the hydatid fluid and the host reaction becomes less intense. At this stage there are three more or less distinct layers: an *inner* one next to the parasite in which the cells are arranged radially and include eosinophile cells and mononuclei, a *middle* one of fibroblasts and eosinophiles which gradually shades into an *outer* band of fibroblasts, which in turn blends with the normal host tissue.

The fate of the parasite is determined by the intensity of this reaction, which varies not only from species to species of host but also between individuals of the same host. If the host reaction is not too intense, the cuticular layer of the parasite becomes thicker and laminated and the trizonal reaction of the host becomes a simple fibrous adventitious cyst. The parasite continues to grow now as a "benign" tumor with an obviously decreased toxicity in its excretions. As it grows, and ultimately because of the basically avascular structure of the adventitia which also grows, the parasite may be starved, become degenerate, and die.

The life and character of the cyst are accordingly determined by two interacting factors, the vitality (and consequent toxicity) of the larva and the intensity of the reaction of the host to both the presence of the parasite and its toxicity. These vary in time as well as in species or strains of parasites and of hosts, or even of kind of host tissue invaded. The reaction of the host to the parasite may therefore have the following effects.

(1) It may contain the parasite with fibrous tissue to form a hydatid pseudotubercle or granuloma. This results in an arrested or still-born parasite.

(2) It may permit the parasite to expand to form a simple or sacculate monovesicular or a multivesicular hydatid which, in either case, may be macrocystic or microcystic and which may be sterile or fertile (according to its age). These types of cyst are normal and healthy. Monovesicular cysts are characteristic of *E. granulosus* types, multivesicular of *E. multilocularis* types, while macrocystic cysts are found in large animals, microcystic in rodents.

(3) It may deprive the parasite of food as the result of the severity of the reaction and ultimately cause its death. Such cysts are abnormal and may be multicystic or pseudoalveolar (which are found rarely in *E.*

granulosus types and are non-metastasizing and benign) or alveolar[2] (which are found in *E. multilocularis* types and are metastasizing and malignant). Such abnormal types, which are more or less moribund or necrotic, have been studied mainly in man and cattle, both unsuitable hosts for *Echinococcus*. Fertile microcystic multivesicular hydatids of the exuberant type produced in rodents by *E. multilocularis* have not been seen in man: only the pathological nearly sterile alveolar form has been reported from him.

Materials and Methods

Primary infections were made in lightly anaesthetized animals by introducing 0.5 ml of a suspension of gravid segments and onchospheres by mouth. The *E. granulosus* (Lebanon) material was kindly supplied by Dr. Calvin W. Schwabe of the Department of Tropical Health, Schools of Public Health and Medicine, The American University, Beirut, Lebanon, who collected the cestodes from a dog which had been fed cystic material of bovine origin. Cysts from reindeer from the Northwest Territories were used to infect a dog and thus provide the *Echinococcus granulosus canadensis* (1) material. Adult *Echinococcus multilocularis sibiricensis* were obtained from two dogs which had been given cysts from cotton rats; the original onchospheres were kindly supplied by Dr. R. L. Rausch of the Arctic Health Research Center, U.S. Public Health Service, Anchorage, Alaska.

Intraperitoneal injection of 0.3 ml of scoleces in penicillin were made on anaesthetized animals in order to establish secondary infections. The scoleces used were collected from *E. granulosus* cysts found in elk and reindeer in northwestern Canada, or from *E. m. sibiricensis* cysts from cotton rats.

Host animals included cotton rats (*Sigmodon hispidus*), voles (*Microtus pennsylvanicus*), golden hamsters (*Mesocricetus auratus*), gerbils (*Meriones unguiculatus*), muskrats (*Ondatra zibethica*), collared lemmings (*Dicrostonyx torquatus*), white mice (*Mus musculus*), chinchillas (*Chinchilla laniger*), white rats, and guinea pigs. In addition, three strains of mice, AKR, DBA/IJ, and C57BL/6J, obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine, were also infected. A vole, naturally infected in Switzerland, was provided by Professor Jean Baer of Neuchâtel, while Doctor A. Neghme of the Departamento de Parasitologia de la Universidad de Chile, presented us with slides of *Octodon* sp., which he had artificially infected with *E. granulosus*. The lemmings were kindly presented to us by Professor K. C. Fisher of Toronto University, and we wish to express our thanks to him and to Professor G. Lubinsky (now of the Department of Zoology, University of Manitoba), who carried out some of the infections.

Results

Primary Infections

Echinococcus multilocularis sibiricensis

Cotton rats.—Of 38 rats infected, 36 became positive. Two weeks after infection the liver is studded with small white cysts; by the fourth week,

[2]In view of the complicated nomenclature which has arisen around this very pleomorphic parasite, the term *alveolar* should be restricted to the *pathological* variant which has been extensively studied in man and *multilocular* should be dropped as a term descriptive of a type of cyst.

large multivesicular cysts have developed but scoleces are either absent or rudimentary. Mature scoleces are present after 7 or 8 weeks and by this time, the liver has usually been almost completely replaced by cystic material which has also spread throughout the adominal cavity. The cyst mass is composed of numerous multivesicular units which average 5 mm in diameter; they are white and translucent, soft in texture, and filled with a gelatinous matrix containing brood capsules and scoleces. In two cases, transparent fluid-filled vesicles as large as 2.5 cm in diameter were found in association with the microcystic structures (Fig. 1). Calcareous granules are present but not in such quantities as are found in cysts in voles.

Gerbils.—Of 20 gerbils infected orally, 4 were positive. The growth rate is slow and cysts with mature scoleces have not been collected in less than 5 months. In three animals, the cyst occurred as a single compact mass which was confined to one lobe of the liver while in one gerbil, two hydatids were found. The liver tissue, exclusive of that directly adjacent to the hydatid, appeared normal. The cyst is yellowish in color and opaque. It is hard in consistency due to the fact that it contains very little fluid and a tremendous number of calcareous granules.

White mice.—Of 12 mice of no particular strain which were infected, five were positive. Fourteen weeks after infection the surface of the liver was studded with small white spots, which were degenerate cysts.

DBA/1J mice.—Sixteen mice were infected. Of the nine mice killed 19 weeks after infection, three contained very small multivesicular masses in the liver. Of the five mice killed after 37 weeks, two were positive. One had a single multivesicular mass and the second, three multivesicular cysts. Each cyst was contained in a separate lobe of the liver and each occupied an area of approximately 1 sq. cm (Fig. 2). These hydatids are microcystic in nature, yellowish in color, and transparent. They were filled with scoleces the majority of which were immature, and a few calcareous granules.

C57BL/6J mice.—Nine mice were infected. Of the six mice killed 19 weeks after infection, one was positive. A very small multivesicular cyst was located on the diaphragmatic surface of the liver. Three mice killed after 24 weeks were negative.

White rats.—Five rats were infected. Three were examined after 16 weeks and two 36 weeks after infection. All were negative.

Muskrats.—Muskrats are readily infected. Growth is slow and after 6 weeks numerous small cysts are present throughout the liver (Fig. 3), which after 5 months is almost completely replaced by hydatid material (Fig. 4).

Echinococcus granulosus (LEBANON)

Cotton rats.—Of three rats infected, two were positive. Eight weeks after infection one rat had one cyst in the liver while the other had three. The hydatids appeared as univesicular fluid-filled vesicles which ranged in size from 1 mm to 4 mm (Fig. 5).

White mice.—Of 13 mice infected, 4 were positive. One mouse, which died after 11 weeks, had four univesicular cysts in the liver, varying in size from 2 mm to 5 mm, and one multivesicular cyst composed of four small vesicles. The remaining mice were killed 18 weeks after infection. Two of

these had cysts in the liver. One had a large univesicular cyst which measured 6 mm in diameter (Fig. 6), while the other had a univesicular and a small multivesicular cyst. A large univesicular cyst, 9 mm in diameter, was found in the right pleural cavity of the third mouse (Fig. 8). A second, smaller cyst was attached to the intercostal muscles directly above the large cyst; in addition, two minute vesicles were observed in the liver. The cysts were filled with fluid, transparent, and sterile.

C57BL/6J mice.—Eleven mice were infected. All were negative when examined 15 weeks after infection.

AKR mice.—Five mice were infected all of which were negative when examined 15 weeks after infection.

White rats.—Five rats were infected. Three of them were examined 15 weeks after infection and two 36 weeks after infection. All were negative.

Echinococcus granulosus canadensis

Cotton rats.—Three rats were infected. All were negative when examined 30, 60, and 90 days, respectively, after infection.

Gerbils.—Four gerbils were infected. All were negative when examined 23 weeks after infection.

White mice.—Eleven mice were infected. All were negative when examined 14 weeks after infection.

DBA/1J mice.—Five mice were infected. All were negative when examined 23 weeks after infection.

C57BL/6J mice.—Five mice were infected. All were negative when examined after 19 weeks.

AKR mice.—Of five mice infected, one was positive. Seven small, fluid-filled univesicular cysts were found in the lungs 23 weeks after infection (Fig. 7).

White rats.—The one rat infected was negative 15 weeks after infection.

Guinea pigs.—Five guinea pigs were infected. All were negative 24 weeks after infection.

Secondary Infections

Echinococcus granulosus canadensis

Cotton rats.—Four rats were inoculated with scoleces from an elk hydatid and three with scoleces from reindeer cysts: all were negative.

DBA/1J mice.—Six mice were infected with scoleces from an elk hydatid: all were negative.

C57BL/6J mice.—Six mice were infected with scoleces from an elk hydatid: all were negative.

White mice.—Of eight mice infected with scoleces from a reindeer cyst, four were positive 32 weeks after infection. In each case, sterile, microcystic vesicles were found attached to the surface of the liver or spleen.

Microscopic Anatomy

In the development of *E. m. sibiricensis* in a "non-resistant" host such as the *cotton rat*, there is a typical but very rapid growth in the liver (Figs. 9–14, 19). It starts with the usual necrotic reaction, which is soon followed

by an accumulation of leucocytes and Küpffer cells. The toxic reaction is not severe, however, although parenchymatous cells outside of the zone of infiltration are degenerating. Giant cells are rare and the predominant cells are polymorphs. After the first week, fibroblasts commence infiltrating the outer edge of the leucocytic zone. Some eosinophiles appear now and increase in numbers and the necrotic zone and degenerating liver cells are being isolated by fibrous tissue from the normal parenchyma. During the second week there is an increased development of the germinal layer which tends to grow into the adventitia with the formation of secondary vesicles. The parasite is now becoming multivesicular and as it develops, its growth overcomes the reticuloendothelial reaction and becomes very rapid; the fibrous adventitia is reduced mainly to thin strands between the vesicles but there is a severe atrophy of the parenchymatous cells as the result of pressure. During the third week, the laminated layer is secreted. Growth is by peripheral herniation of the numerous microvesicles which thus carry with them a thin layer of adventitia.

Scoleces begin to appear about the fifth week in what appear to be brood capsules which sometimes fill the microvesicle; sometimes the vesicle may be partitioned into three or four sections by growth from the germinal membrane. Ultimately, these scoleces—which are always invaginated—almost fill the microvesicles together with a large number of minute, spherical, calcareous bodies. Growth continues until the death of the animal in some 8 weeks or more. The liver is relatively enormous and, while the parenchyma is almost entirely eliminated, represents more than half the total mass of the rat, while the micropolyvesicular cyst is more than half the mass of the liver.

In a few rats, metastases were found in the lungs.

In general, the individual vesicles are very small—under 10 mm—but sometimes larger ones appear. In these, there is a single layer of brood capsules, containing single scoleces, along the outer margin of the vesicle, which has a well developed cuticular layer, with a single layer of elongate fibrous tissue cells on the outside, just within the capsule of the liver (Figs. 15–16, 17).

Cotton rats occasionally show the degenerate alveolar form, usually shading into a sterile, nearly normal form, which at its opposite margin may contain a few scoleces arising from a quite well-developed germinal membrane (Figs. 18, 20–21). The cuticular membrane is also well developed at this point and remains so in the larger microvesicles. In the smaller ones, however, it is thin or even absent and is surrounded by a heavy avascular, poorly staining adventitia. None of our cotton rats, however, showed actual necrosis. In some, the parasite mass is completely sterile, although part is quite similar to the fertile variety but with heavier adventitia.

There is, accordingly, an almost complete transition from the exuberant, fertile, multivesicular cyst to the degenerate, tumor-like alveolar form, and this transition can be found in the same animal.

Infection with onchosphere of *E. granulosus* (Lebanon) produced in the liver a small, spherical cyst with a well-developed germinal layer and a developing cuticular layer. There were no budding brood capsules. The host reaction was relatively slight and appears to result from pressure rather than from toxicity (Fig. 39).

Secondary infections *E. m. sibiricensis*, produced by parenteral inoculations of minced primary (or secondary) cysts subcutaneously or in the abdominal cavity, develop slightly differently. There is little fibrous tissue but more cuticular tissue (of parasite origin) is produced to support the parasitic mass. Central vesicles showed a tendency to degenerate while some cysts became detached and trapped in the omentum. Budding begins in 48 hours and by the end of 14 days the buds have become vesicularized. Scolex formation may be complete in as little as 3 weeks after inoculation in initial implants but after a series of subinoculations scolex formation was less luxuriant and acephalocysts were not infrequent.

There is no humoral eosinophilia and local eosinophiles, although present, are never common. The predominant leucocyte appears to be the heterophile.

In *voles* the growth is even more active than in cotton rats and although otherwise similar in these smaller animals, the cyst may exceed the weight of the host in less than 2 months after infection. The vesicles, however, are more variable in size and have fewer scoleces than in those of cotton rats; brood capsules often contain only one scolex, and in the larger cysts, the scoleces are often arranged around the periphery. The host reaction is not excessive and the cuticular layer is relatively thick and well formed. On the outside of the layer there is a layer of degenerating leucocytes, some two to eight cells thick with a relatively avascular fibrous tissue outside; in turn, this is in contact with atrophying tissue which shades into normal liver tissue.

In a vole received from Dr. Rausch, much the same appearance was seen but growth was more exuberant and the cuticular layer was rather more obvious. Adjacent vesicles were always separated from each other by a very thin layer of fibrous tissue. Calcareous granules were prominent as was also the case in cotton rats and local voles. The host reaction was very slight and the parasite was growing rapidly.

A European vole, received from Prof. J. Baer of Neuchâtel, was naturally infected with *Echinococcus multilocularis multilocularis*. This vole was taken in Switzerland. It tended to show larger microcysts, some fertile, some sterile and degenerate. The fertile cysts contained scoleces only at their periphery, arising from a thin germinal membrane. This cuticular layer was thinner than usual and the fibrous capsule thicker than normal. Degenerate areas were small but were approaching the alveolar type in appearance.

Gerbils have been infected successfully with onchospheres per orum but are more susceptible to percutaneous infections with scoleces. Growth is very slow and chronic and the infection can be long-lasting; a considerable amount of liver tissue remains unaltered after several months. The micropolyvesicular cyst is fertile but with an excess of calcium corpuscles. The cuticular layer is quite well developed and is surrounded by a layer of leucocytes which, in turn, is encapsulated in a rather dense fibrous adventitia.

Yamashita *et al.* (25), who infected gerbils (*Meriones unguiculatus*) orally, found that they were good hosts with a feeble resistance to the parasite. Scoleces took between 60 and 90 days to develop. As late as 200 days after infection, tissue reaction was still slight and regressive changes were observable.

The *muskrat* is a "good" host for *E. m. sibiricensis* but the parasite is very slow growing, more so apparently than found by Rausch and Schiller (17).

Some cysts are of the typical *multilocularis* type as seen in the cotton rat, fertile and full of brood capsules containing four or five scoleces. There is a tendency for the vesicles to be larger, however, and in this case the brood capsules are massed along the periphery and particularly in indentations where loculi occur (Figs. 31–32). The cuticle is substantial and complete and the germinal layer thick and active. Herniations occur and the host reaction is normal. Liver destruction is slower than in cotton rats but faster than in gerbils. In some cases, however, the mass is definitely alveolar (Figs. 30, 33) with numerous sterile microvesicles enclosed in a poor cuticle. Occasionally, where the cuticle is better formed and the reaction less active, in vesicles at the edge of the liver, a few brood capsules can be seen. Elsewhere, however, the vesicles are sterile and surrounded by heavy fibrous tissue into which extensions of parasite cuticle surrounding caseated necrotic material can be seen. This alveolar type of cyst is located at the periphery of the liver but appears to be gradually extending inwards in some places although restricted in others.

In the collared lemming infected with *E. m. sibiricensis* the earliest lesion was a simple spherical vesicle (Figs. 22, 25) with a very thin cuticle and a quite extensive reaction. There was no sign of growth within the vesicle and it is difficult to say whether this would develop into a fertile type of cyst or become alveolar.

A slightly older infection showed a relatively large monovesicular cyst with an active germinal layer and a definite cuticular layer. There were a number of brood capsules originating on the germinal layer but no herniations. The reaction consisted of a layer of more or less necrotic cells next to the cuticle; this, in turn, was followed by well-developed fibrous tissue; on the outside and shading into the liver substance was a thin layer of leucocytes.

In other vesicles in the same animal, however, a rather more advanced development could be seen (Figs. 26–27). Herniation to multivesiculation had commenced and small brood capsules, containing developing scoleces, were present but few in number. The reaction was considerable and of a typical trizonal type and was more intense than in that observed with the young cyst.

In close proximity to the larger microvesicle but separated from it by normal liver parenchyma the alveolar type (Figs. 22, 24) was developing. In a dense mass of amorphic fibrous tissue lay a number of dead vesicles, some necrotic and filled with a caseated material while others showed remnants of the parasite. The reaction at the edge of this lesion was quite intense in parts but quiescent in other portions of the same cystic mass (Fig. 23).

In older lesions in other lemmings, the alveolar condition had developed still further, although single, relatively large monovesicular but sterile cysts were present in the same liver. Others, with a germinal layer and a thin cuticular layer, showed small buds which were probably developing brood capsules while a few young scoleces were also present (Figs. 27–29).

These reactions resemble those described by Rausch and Schiller (17) for Varying lemming (*Dicrostonyx torquatus*) but are more varied and cysts are at least partly fertile.

In the *guinea pig* and the *rabbit* neither primary nor secondary infections

have taken place with *E. m. sibiricensis*, although rabbits are said to be easily infected (6) secondarily by *E. granulosus* and have occasionally been infected by onchospheres (2, 4). Pérez Fontana has also reported the development in rabbits of the alveolar variety following the intraperitoneal injection of pieces of membrane of *E. granulosus* recovered from sheep (13).

Oral infection of white mice has given very variable results with *E. m. sibiricensis* and lesions in the same liver have varied from relatively large, feebly fertile microvesicles of almost normal type to an alveolar type, sterile, with a poorly developed cuticular layer and a dense old fibrous adventitia quite sharply demarcated from the liver substance. In the fertile portions of the cyst, the host reaction is well developed while the cuticular layer of the parasite is poorly developed.

In a young white mouse of no particular strain, infected 70 days previously with *E. m. sibiricensis*, an alveolar condition was already beginning to develop. There was a mass of vesicles embedded in a connective tissue matrix which was still proliferating actively at its margin. Some vesicles contained cuticle and germinal membrane, while those at the edge of the mass showed the typical trizonal reaction.

In the same liver was a single, simple vesicle with a thin germinal layer, a thin cuticular layer, and a relatively intense reaction to its presence (Fig. 34).

In a 125-day infection, the alveolar condition (Fig. 35) had continued to develop, the connective tissue matrix had become almost amorphic, and the host reaction had become less intense. Remnants of parasite membrane could still be seen in the microvesicles. However, lobulated fertile microvesicles were also present outside of the alveolar mass and brood capsules and scoleces were developing. At the junction between the parasite and liver, the reaction was still considerable.

In other infections with *E. m. sibiricensis* (Fig. 36) the liver was very extensively invaded: part was filled with a mass of microvesicles in a poorly staining matrix of fibrous tissue. These vesicles contained relatively small numbers of developing brood capsules and scoleces.

In secondary infections in white mice with *E. m. sibiricensis* between half and three-quarters of the inoculations were successful, although growth was very slow and uncertain, and while scoleces were produced, sterile cysts were common.

Oral infection with onchospheres of *E. g. canadensis* in one AKR mouse produced small cysts (Fig. 42) in the lungs; the liver was normal. It will be recalled that the cyst of this variety appears to occur only in Cervidae (and man) and is invariably found in the lungs of deer. In some hundreds of deer (reindeer, caribou, elk, moose) examined no live hydatids have been found in the liver. The cysts here, although sterile, seemed alive and healthy.

White mice infected orally with onchospheres of *E. granulosus* of Lebanon origin produced an infection which was very similar to that shown in the chinchilla. The parasite, however, was better developed; the fibrous cyst was well formed and discrete but less consolidated, and it shaded gradually into liver parenchyma (Figs. 37–38).

Using *young* white mice (48 days old or younger) of a strain received from the U.S. Naval Medical Research Unit No. 3 in Cairo, Schwabe and his

colleagues (19) in Lebanon were able to produce secondary echinococcosis by the injection of a suspension of scoleces of *E. granulosus*. The result was a mass of small cysts, up to 7 mm in diameter, with laminated and germinal layers but no brood capsules after 3½ months; traces of the original scolex were visible. Macroscopically, the appearance is very similar to that of a secondary infection with *E. multilocularis*. However, the infection was still a peritoneal one although a few cysts were in the liver, having been surrounded by parenchymatous tissue which had replaced the initial inflammatory tissues (heterophiles and fibroblasts).

Golden hamsters were found by Rausch and Schiller (17) to be unsuitable hosts for *E. multilocularis* and lesions showed a necrotic center and a thick band of connective tissue surrounding them. Using albino hamsters, Yamashita (25) had similar results.

We infected two standard hamsters orally with *E. multilocularis* and in one there was a single monovesicular cyst with a poor germinal layer, a thin cuticle, and a considerable host reaction of the usual trizonal type.

In another hamster, infected with onchospheres from *E. g. canadensis*, a simple degenerate cyst with a much folded cuticular layer but no visible germinal layer was found. Fibrous tissue had spread between the folds of the cyst in addition to forming a definite fibrous capsule. Parts of the parasite were commencing to caseate (Fig. 43).

In the *chinchilla*, no infection took place with rodent hydatid, but two minute dead cysts were found in the liver and lungs after the feeding of *E. g. canadensis* (Figs. 40–41) eggs. The parasites, however, were dead and folded on themselves, surrounded by a firm fibrous adventitia sharply separated from normal host tissue.

Two slides of liver cyst from *Octodon* (Fig. 44) (a South American rodent) infected experimentally in Chile with onchospheres of *E. granulosus* (of sheep origin) sent to us by Prof. Neghme. The cyst was of the normal *granulosus* type with brood capsules each of which contained a number of scoleces. There were well-developed germinal and cuticular layers separated from the liver by a wide band of almost amorphic fibrous tissue, on the outer margin of which was a small accumulation of leucocytes. Many brood capsules containing numerous scoleces were in the center of the cyst.

Discussion

Cotton rats, voles, gerbils, muskrats, and mice of the DBA strain were found to be suitable hosts for primary infections with *Echinococcus multilocularis sibiricensis*, but in varying degree.

Although the cotton rat shows the greatest degree of susceptibility, there are several disadvantages in using this animal as the sole laboratory host, if a continual supply of cystic material is required. There is virtually no host resistance to the parasite; the cyst grows rapidly and usually destroys the liver and spreads throughout the whole animal, frequently killing it before the scoleces have matured. It is also extremely susceptible to murine salmonellosis. Gerbils, on the other hand, are semiresistant. The parasite does not metastasize rapidly and while only 25 to 50% of the infected animals are positive it is considered to be a good reservoir host for maintaining

Echinococcus multilocularis. One-third of the DBA strain of mice were positive, but development was very slow and it was 6 months after infection before scoleces were present. This differs from Yamashita's (25) results for he reported that scoleces developed in DBA mice $1\frac{1}{2}$ to $2\frac{1}{2}$ months after infection. His work on the receptivity of mouse strains, however, must be considered of fundamental importance.

The cysts which developed in white mice were partly degenerate, a condition which may be considered as being midway between complete susceptibility and complete resistance. It is suggested that the success or failure of previous investigators to establish infections in this host was determined by the genetic constitution of their particular stock.

Secondary infections of *E. multilocularis* in cotton rats, gerbils, and white mice are, as reported by Lubinsky (11), readily established and serial transfers provide a convenient method of maintaining the parasite without the hazards involved in handling *Echinococcus* infective to man. In vitro studies, however, have shown that material of secondary origin behaves differently in culture from material derived from primary infections (22). Lubinsky (11) noted morphological changes in the scoleces as a result of the secondary type of development and it is assumed that the parasite also alters physiologically.

Secondary infections, resulting from the subcutaneous or intraperitoneal injection of scoleces, tend to be successful in a wider variety of hosts than are primary infections, probably because of avoidance of the reaction of the liver.

The relationship between the parasite and the host differs not only with the species of cestode but with the species of rodent. In the case of *Echinococcus multilocularis sibiricensis* in some hosts, there is exuberant growth in which the parasite overcomes the apparently feeble host resistance and reaches an enormous size. Such parasites develop great numbers of scoleces and ultimately destroy the host by their very volume. If the host remains alive sufficiently long, however, degeneration of the inner parts of the massive growth tends to occur with central necrosis of the parasite. In other cases, the host resistance is greater and ultimately comes to contain the parasite in an almost avascular, amorphic adventitious cyst. In the interstices of this fibrous mass, poorly developed microcysts occur; most are without scoleces and obviously moribund or degenerate. This is the equivalent of the so-called alveolar conditions recorded so far only from man, an obviously unsuitable host from which the exuberantly successful form of *Echinococcus multilocularis* seen in rodents has never been reported.

In this condition in man, there is a mass of minute vesicles of irregular shape, with degenerated hyaline membrane and little or no fluid. The cavities are enclosed in a heavy, avascular, more or less amorphic fibrous stroma and are nearly always sterile. Only a very occasional microvesicle contains scoleces, and the germinal layer, when evident, is thin and weak. The cavities extend peripherally, with necrosis and cavitation in the center of the lesion. Growth appears to be slow judged from the thickness of the fibrous tissue, the necrosis, the occasional calcification, and the absence of evidence of an active tissue reaction.

While in man an identical picture is occasionally produced by *Echinococcus granulosus*, the underlying causes are not clear. In such cases, this pseudo-

alveolar cyst has always been recorded from elderly subjects. Unlike true alveolar, however, no metastases have been recorded. The comparative histology of these alveolar-like cysts in rodents suggests that the cuticular membrane plays a critical part in the type of reaction and that the hydatid fluid excreted across it into the liver determines the intensity of the host response. There may be a continuous production of a fluid of relatively weak toxicity against which the host reacts slowly but continuously or the fluid may be more strongly toxic causing a faster but equally severe reaction of the reticuloendothelial system. In either event, the effect on the parasite is adverse, resulting in an almost or completely sterile series of microvesicles. If the host response to the hydatid fluid of *E. m. sibiricensis* is poor, as is the case in cotton rats and voles, the growth is exuberant and the host is killed.

An intermediate reaction, such as is seen in muskrats, produces a much slower but partly fertile growth with some development of the alveolar condition in the older lesions. In other rodents, such as some mice, the reaction is even less favorable, while in still others, such as chinchillas, white rats, and hamsters, the parasite is destroyed almost as soon as it becomes lodged in the liver and forms a hydatid pseudotubercle.

Degenerate cysts with a thick adventitia tend to produce an excess of cuticular substance which lies folded up within the adventitia along with caseated debris. This excessive growth of cuticle at the expense of the germinal membrane suggests that the parasite, while still alive, is unable to develop properly through lack of suitable nourishment and that its activities are confined to attempts to protect itself from the host reaction by the development of excessive cuticle. Such changes have been noted in *E. granulosus* cysts in some rodents and to a lesser extent in the seminecrotic vesicles of the alveolar-like cysts of *E. multilocularis*.

Infections with onchospheres of *E. granulosus* have been successful in the liver and lungs of some rodents, thereby confirming the previous work of Dévé (2, 3, 4) and Yamashita (23). In some cases, however, the parasite has been killed and encapsulated while in others, although microvesicular, it appears to be developing normally and it is interesting to speculate whether, if given sufficient time, they might have produced scoleces. It is also interesting to note that in two instances multivesicular cysts were found. This leads us to wonder whether multivesicular development is actually a function of a specific species of *Echinococcus* or merely the manifestation of a poor host-tissue reaction.

In the past, success or failure in infecting rodents with *Echinococcus* eggs was used as a criterion in differentiating between *E. multilocularis* and *E. granulosus;* this criterion is no longer valid.

There is some evidence that the *E. granulosus* occurring in northern Canada is a specific biological strain, including the fact that the cysts have a definite predilection for the lungs; this is true in the normal intermediate hosts (elk, reindeer, moose, and caribou) as well as in man. In the one mouse and the chinchilla which were positive after being infected with eggs from this source, the cysts were also found in the lungs. Furthermore, secondary infections were unsuccessful; small sterile cysts developed in only one group of mice.

Another finding of interest is the fact that in some rodents there is a very

heavy development of calcareous corpuscles within the microvesicles of *E. multilocularis* whereas in other species, such corpuscles are absent or almost so. Also, in some hosts, brood capsules, although always small, contained several scoleces whereas in others they had a single scolex. In larger vesicles, these brood capsules tend to occur only along the germinal membrane (as they do in *E. granulosus*) while in the more exuberant growths, as seen in cotton rats, they present an almost solid mass of scoleces completely filling the vesicles and often apparently free from the germinal membrane.

Further experimentation with *Echinococcus* in rodents may yield more specific information on host–parasite relationships.

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NOTE: Figs. 1–44 follow.

PLATE I

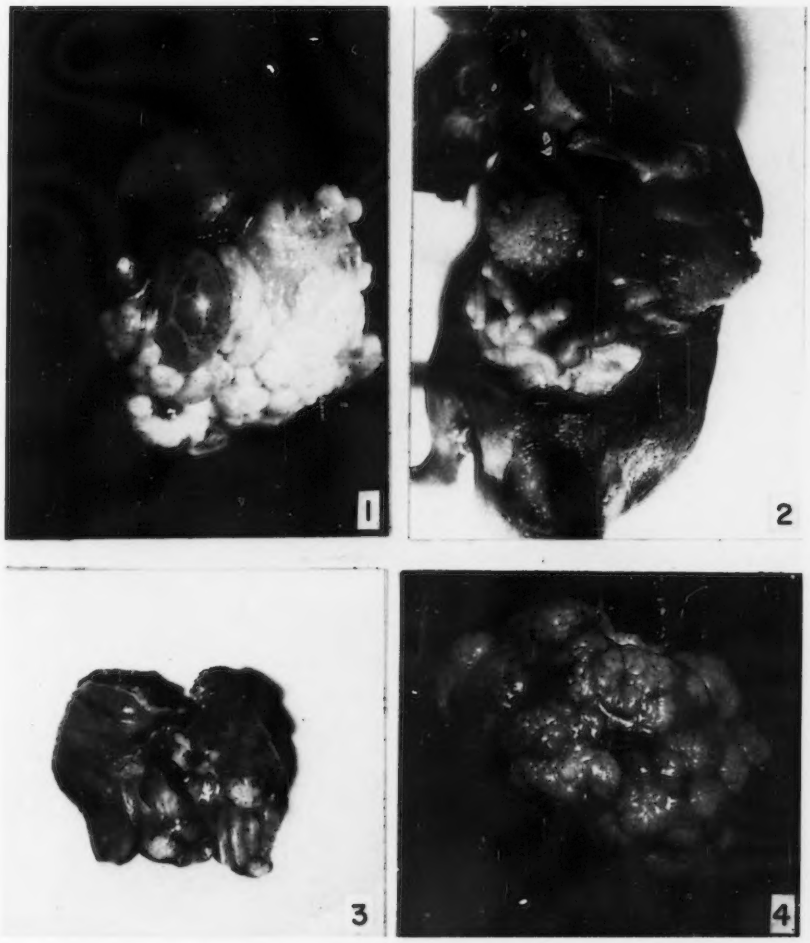


FIG. 1. *E. multilocularis sibiricensis:* primary cysts from cotton rat liver.
FIG. 2. *E. m. sibiricensis:* primary cysts in liver of DBA strain mouse 37 weeks after infection.
FIG. 3. *E. m. sibiricensis:* primary cysts in liver of a muskrat 6 weeks after infection.
FIG. 4. *E. m. sibiricensis:* primary cysts in liver of a muskrat 5 months after infection.

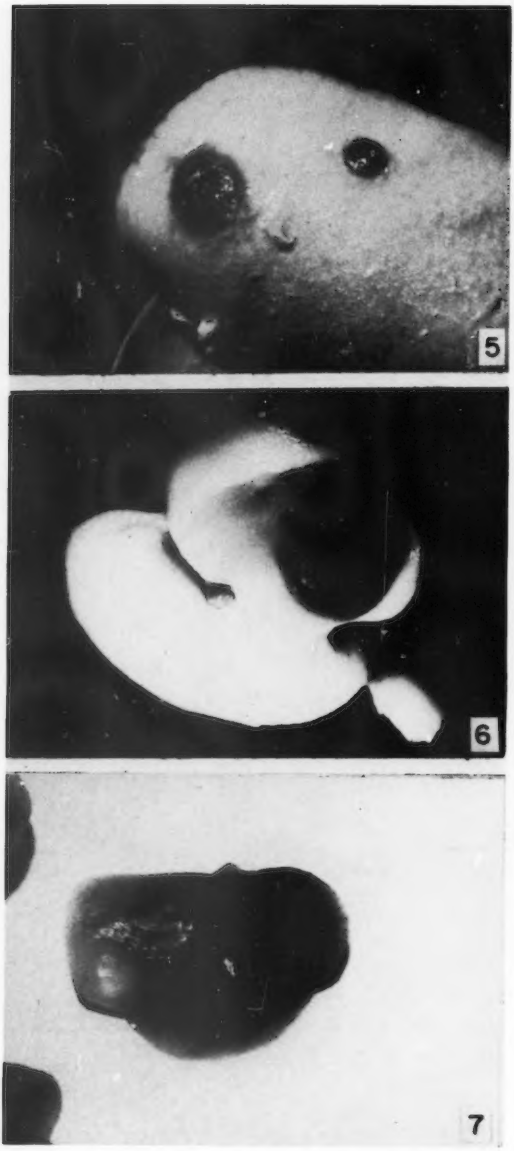


FIG. 5. *E. granulosus* (Lebanon origin): primary cysts in liver of cotton rat 8 weeks after infection. FIG. 6. *E. granulosus* (Lebanon origin): primary cyst in liver of white mouse 18 weeks after infection. FIG. 7. *E. g. canadensis:* primary cyst in lung of AKR strain mouse 23 weeks after infection.

PLATE III

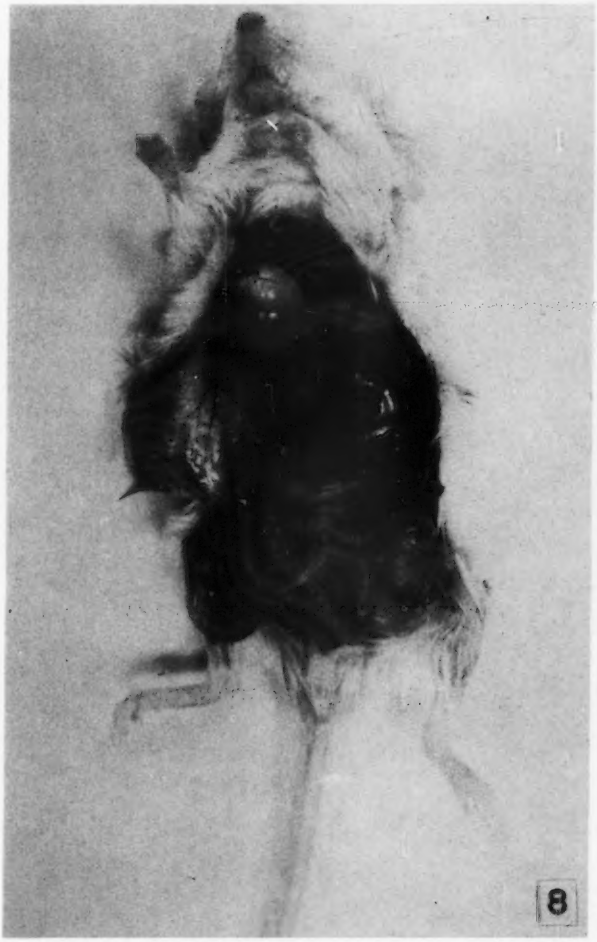


FIG. 8. *E. granulosus* (Lebanon): primary cyst in pleural cavity of white mouse 18 weeks after infection.

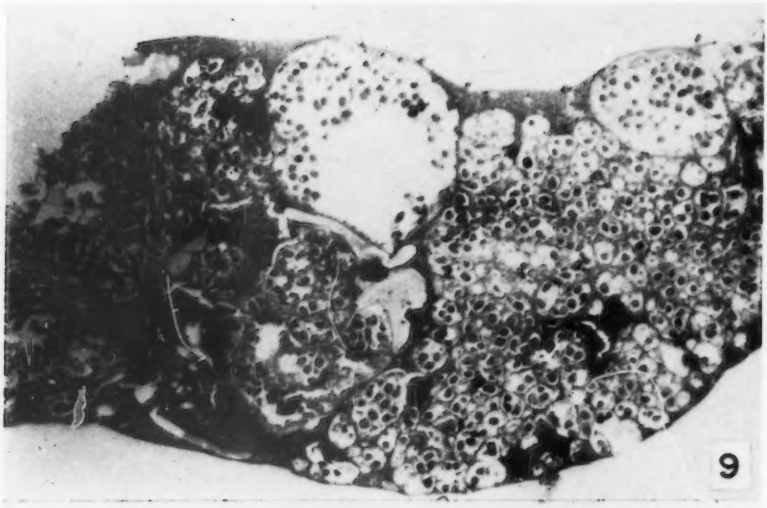


FIG. 9. Low power view of section of liver of vole experimentally infected with *E. m. sibiricensis*, showing extensive invasion of tissue in numerous small vesicles as well as two comparatively large ones. This is the typical picture in both cotton rats and voles. (× 12)

FIG. 10. A high power view of Fig. 9, showing the sharp demarcation between parasite and host tissue. (× 30)

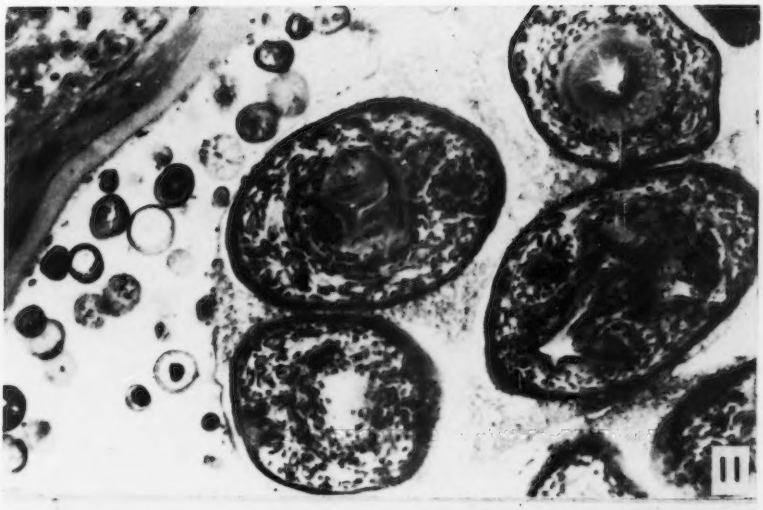


FIG. 11. A portion of Fig. 9, showing a brood capsule, four scoleces, with innumerable calcium corpuscles, a thin germinal layer, and a relatively well-marked cuticular one. (× 300)

FIG. 12. Junction between four microvesicles showing an islet of host tissue spreading between the cuticular walls of the vesicles. (× 480)

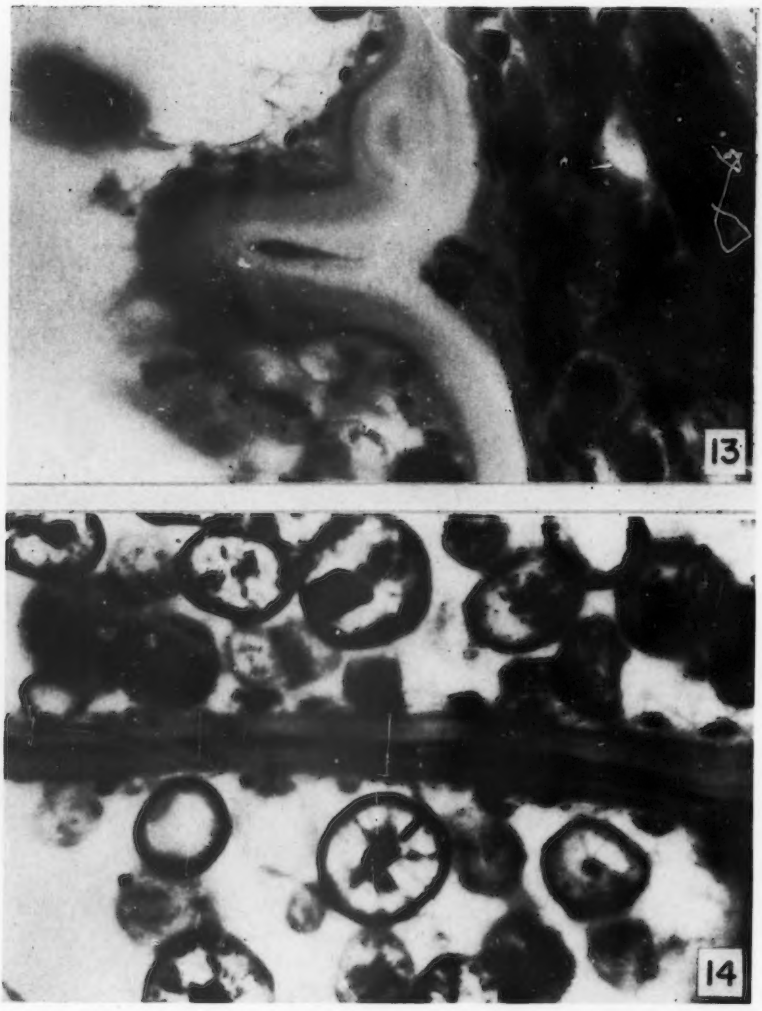


FIG. 13. Infiltration of a herniation of a vesicle with fibroblasts already entering the junction. (× 750)

FIG. 14. Junction between two vesicles showing the single layer of fibrous cells separating them. The circular bodies are calcareous corpuscles. (× 750)

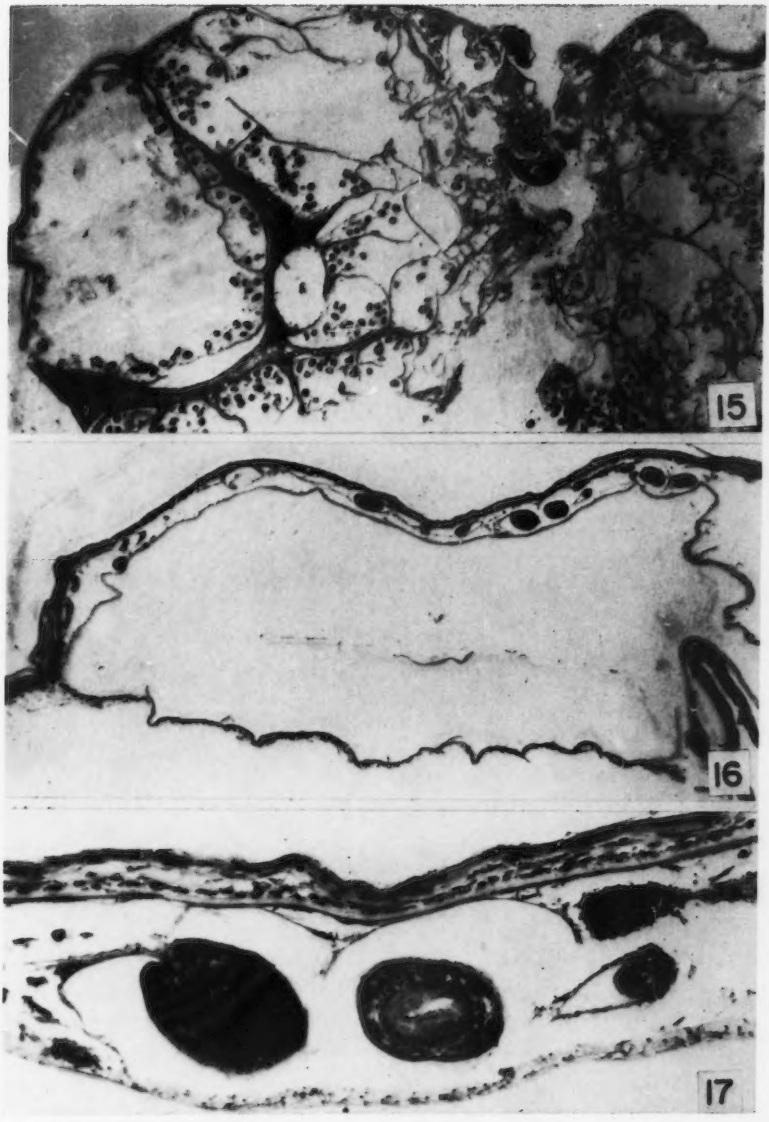
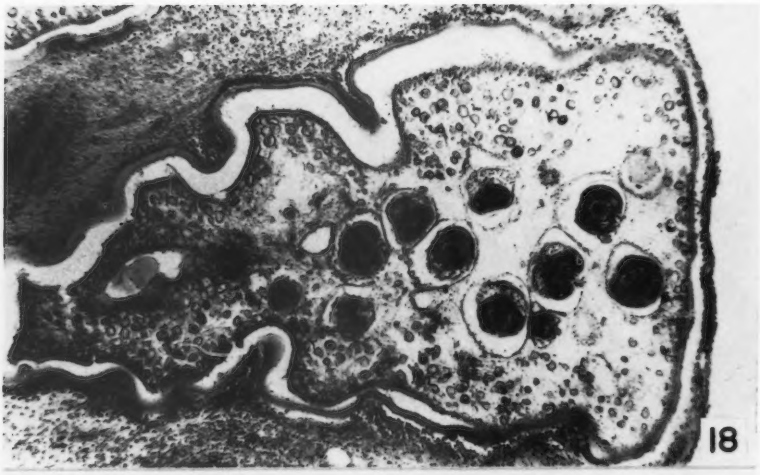


FIG. 15. A low power view of a less successful invasion of a vole in which relatively large vesicles are seen with margination of the brood capsules. A slightly necrotic region is seen between two fertile areas of vesicles (× 12). FIG. 16. This shows the occasional large vesicle seen in both species of hosts, in which the scoleces are peripheral. In this case (from a cotton rat) they were present only on the margin of the vesicle next to the liver capsule (× 30). FIG. 17. This shows in more detail the portion of this vesicle with the single row of brood capsules, most with a single scolex. The parasite membranes are well developed but the host reaction is slight (× 200).



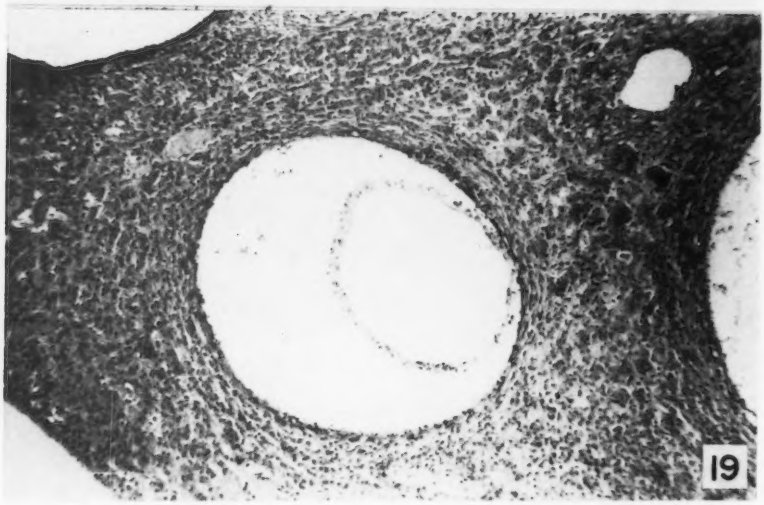
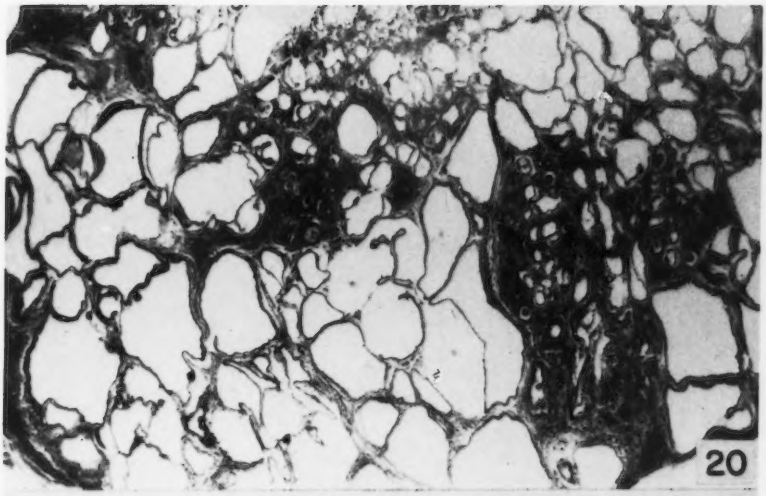


FIG. 18. This shows a large cyst in a cotton rat with a heavy development of calcareous bodies and few scoleces. The cuticular layer, although well developed, is becoming indented and the host reaction is greater than usual. (× 75)

FIG. 19. A young cyst in the liver of a cotton rat in which the alveolar form was developing. There is already both a well-developed germinal layer and a considerable degree of host reaction but the cuticular layer is poorly developed. (× 75)



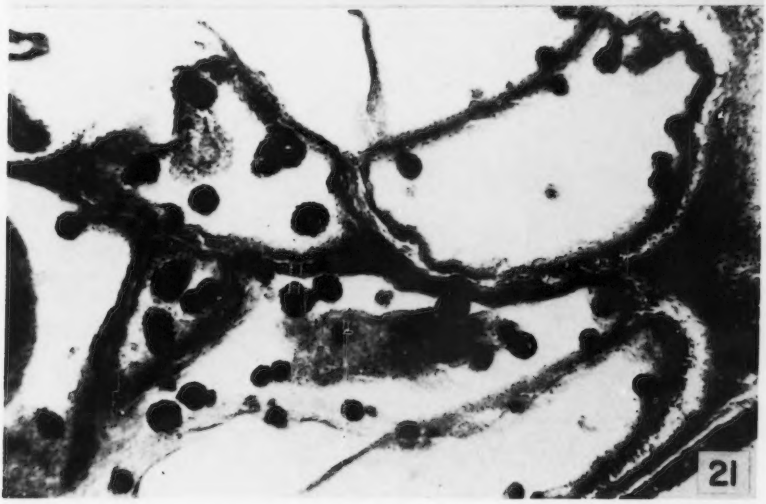


FIG. 20. A portion of the same liver as Fig. 19 showing a sterile mass of alveolar type cysts. Actual necrosis has not been seen in these rodents. (× 30)

FIG. 21. Another portion of the same liver adjacent to that in Fig. 20, showing the development of a small number of scoleces. The host reaction is greater than usual. (× 75)

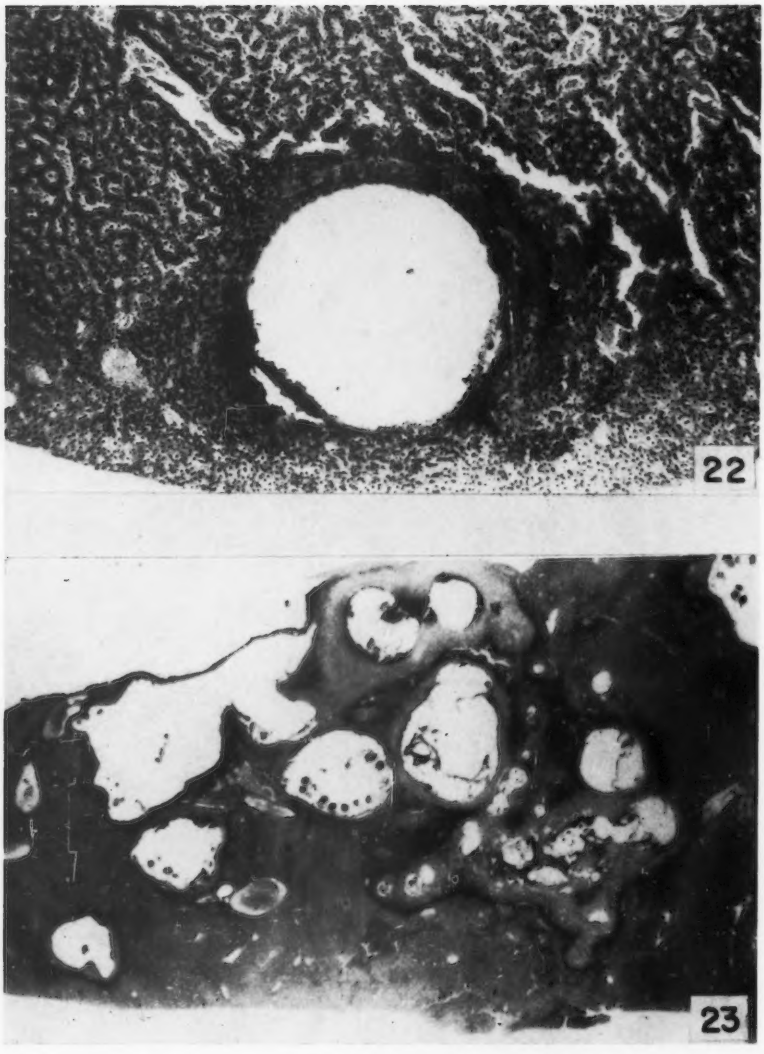
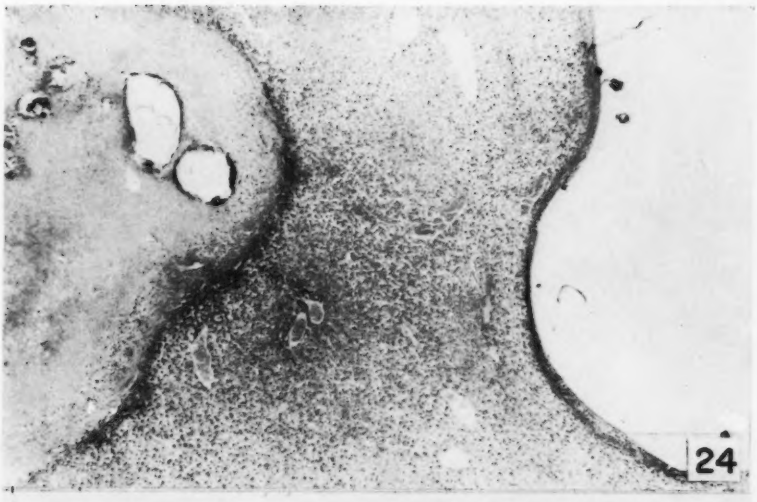


FIG. 22. An early lesion in the collared lemming infected with *E. m. sibiricensis*, showing a developing cyst with a well-marked host reaction to its presence. (× 75)

FIG. 23. An alveolar mass in the lemming showing a well-defined host encapsulment, the beginning of actual necrosis in some vesicles, but a few developing scoleces in others. (× 12)



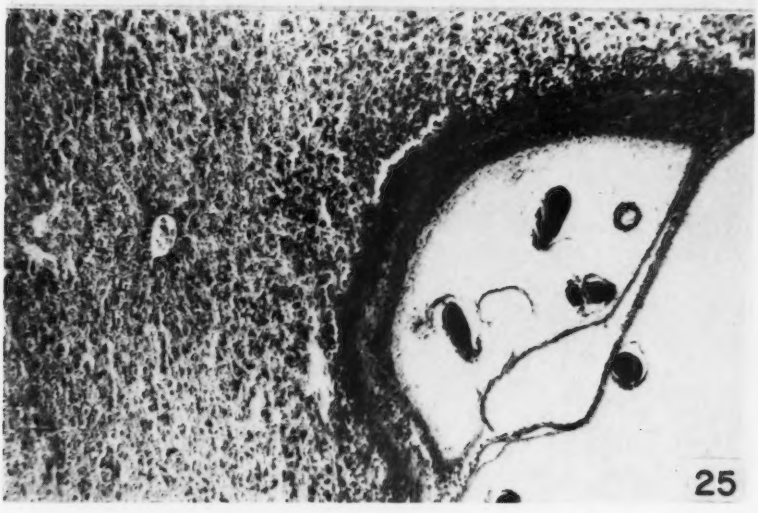


FIG. 24. An almost avascular necrotic area in the liver of a lemming may exist and be almost contiguous with a semifertile cyst. The reaction to the necrotic area is much more severe than to the relatively large semifertile vesicle. (× 30)

FIG. 25. A young cyst in the lemming, although fertile, provokes a considerable host reaction of the trizonal type. The commencement of rather poor secondary vesicles can still take place. (× 75)

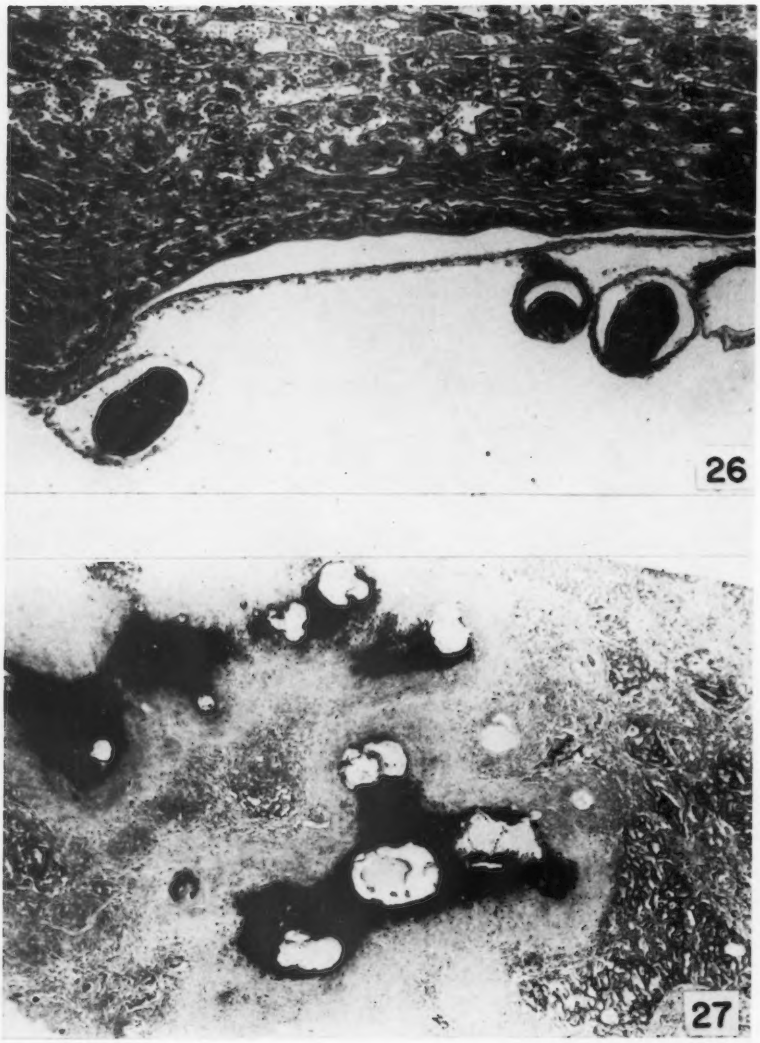
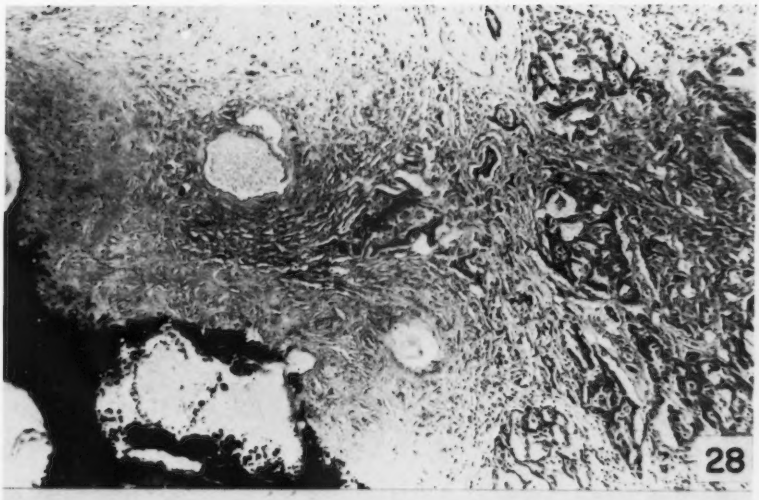


FIG. 26. In such a cyst the cuticle and germinal layer appear about normal and the brood capsule appears to develop a single scolex. (× 200)

FIG. 27. In old infections in the lemming, the avascular tissue surrounding the parasite becomes dense. The parasite becomes necrotic and caseated and central vacuolation occurs. (× 30)



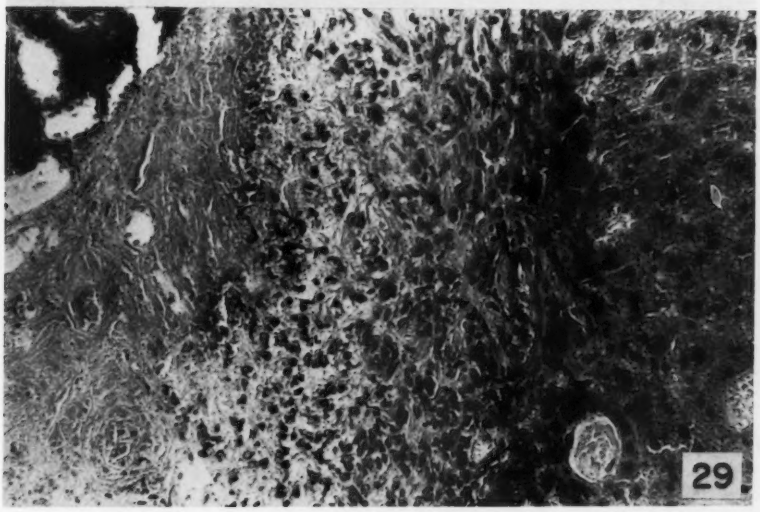
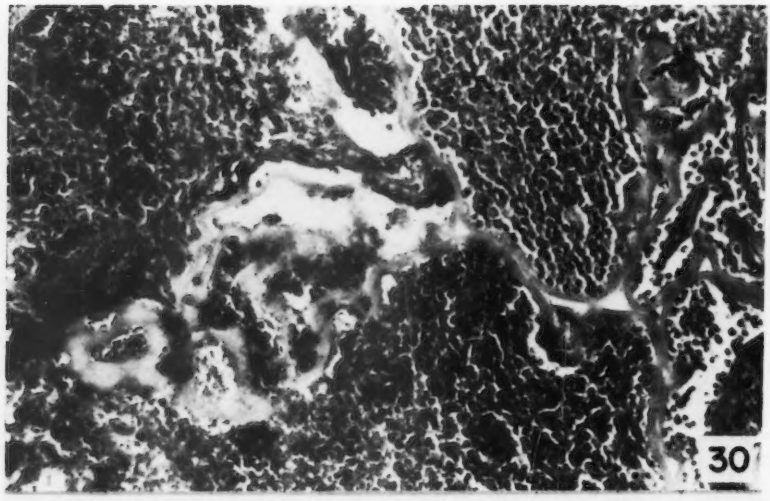


FIG. 28. The edge of such a lesion as shown in Fig. 27 shows the dense fibrous tissue with a few isolated patches of liver parenchymatous cells at the periphery. The parasite is completely degenerate. (× 75)

FIG. 29. The reaction at the edge of the alveolar mass is still extensive but the transition to normal liver cells is relatively sharp. (× 200)



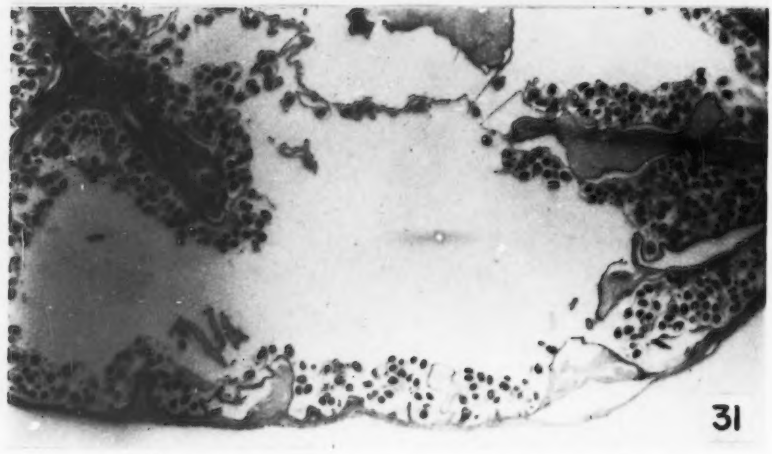
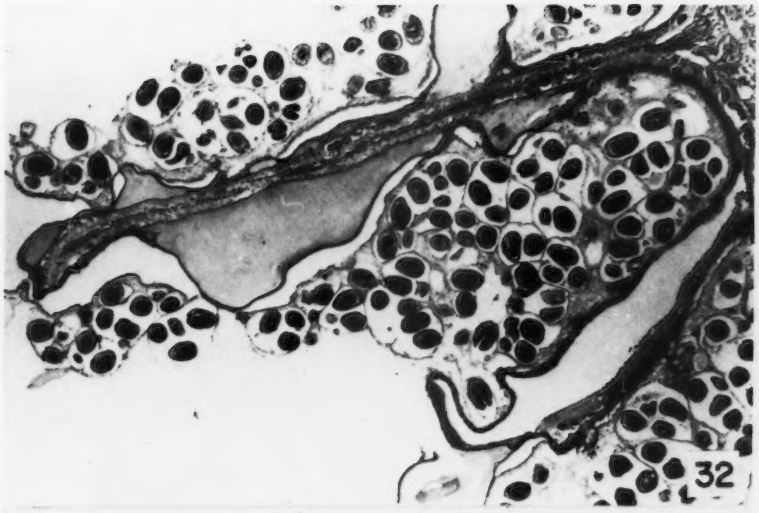


FIG. 30. Infiltration of liver in an old infection in the muskrat with *E. m. sibiricensis*, showing the invasive nature of the parasite. (× 200)

FIG. 31. A cyst from the outer part of the liver of the muskrat showing the accumulative scoleces around the periphery and their concentration in those portions of the cyst which have become partly cut off. (× 12)



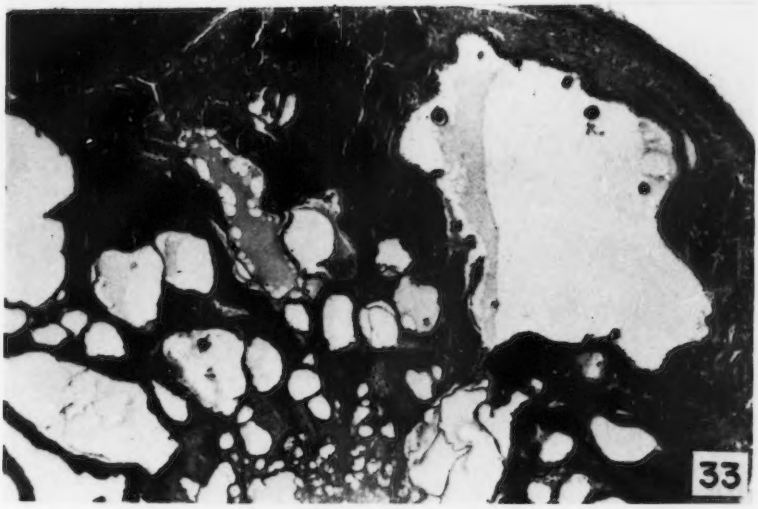


FIG. 32. A portion of the cyst in Fig. 31 showing this accumulation. There is an absence of the calcium corpuscles so common in cotton rats and voles. The parasite membranes are well developed but the host reaction is a little more intense than in the cotton rats. (× 30)

FIG. 33. A degenerate, alveolar portion of the same cyst with intense fibrosis and numerous sterile vesicles. One vesicle, however, contains a very few poorly developed brood capsules and scoleces. (× 12)

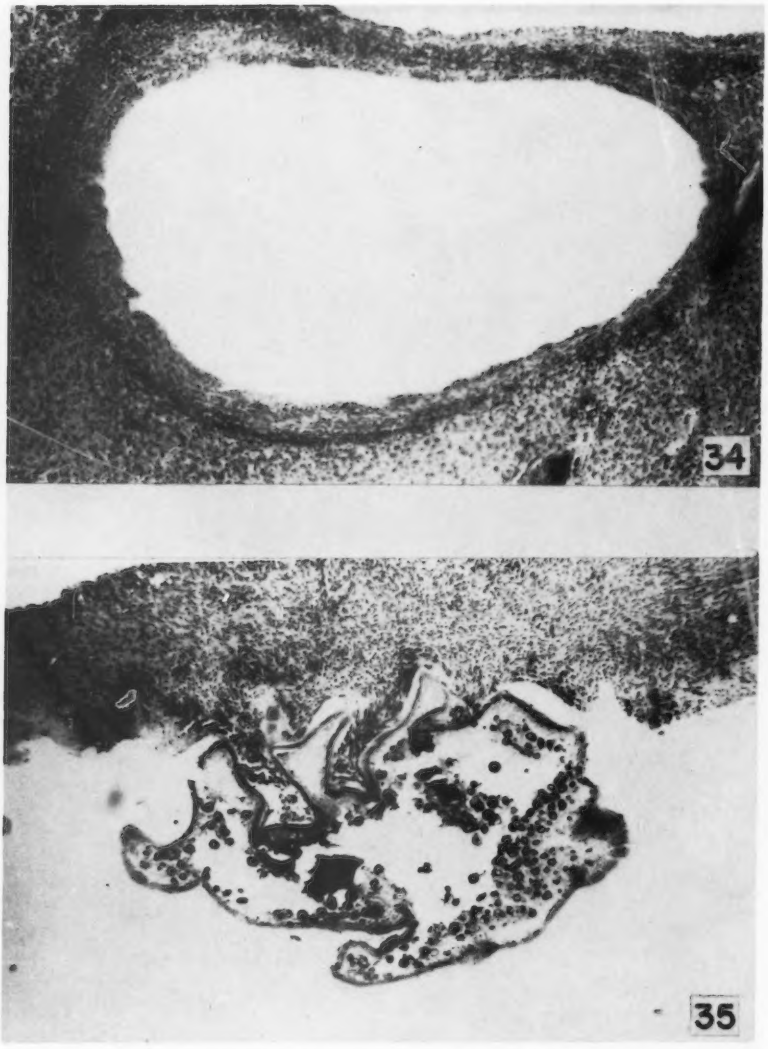


FIG. 34. In the white mouse, 70 days after infection with *E. m. sibiricensis*, a well-defined monovesicular cyst developed. The trizonal reaction was considerable, the cuticle was poorly developed and the germinal layer was apparently still alive. Elsewhere in the liver of the same animal an alveolar condition was also beginning to develop. (× 30)

FIG. 35. In a white mouse, infected orally with onchospheres from the same dog and killed 125 days later, a well-developed cyst was found containing numerous brood capsules. (× 75)

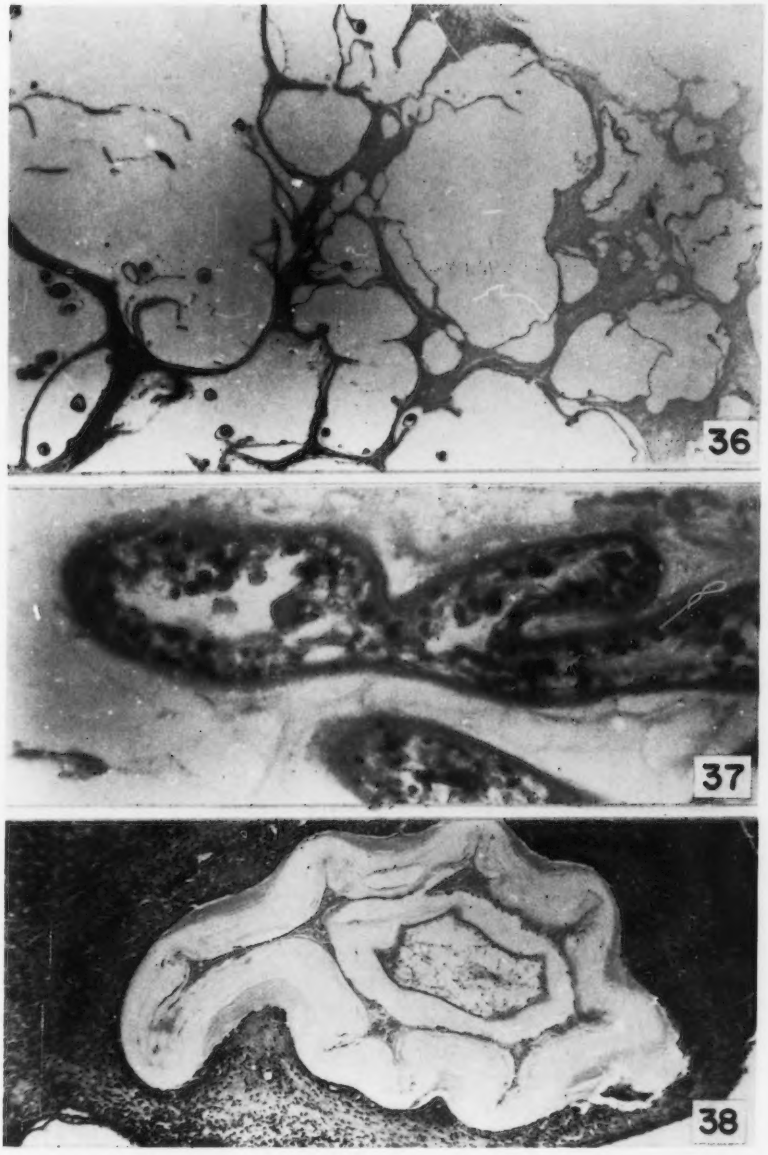


FIG. 36. More common was a mass of dead or nearly dead microvesicles, infiltrating large parts of the liver. The dead vesicles were embedded in poorly staining fibrous tissue while the living ones, which contained a few scoleces, were surrounded by a more active fibrous tissue (× 30). FIG. 37. Portion of a much convoluted cyst in liver of a mouse infected with onchospheres of *E. granulosus* (Lebanon); cyst on outer margin of the liver and both layers of the parasite apparently alive (× 480). FIG. 38. Monovesicular cyst in the liver of a mouse infected with onchospheres of *E. granulosus* (Lebanon). The cyst is apparently dead but there is a great development of cuticular membrane surrounded by fibrous tissue. This appearance closely resembles that frequently seen in large mammals (× 75).

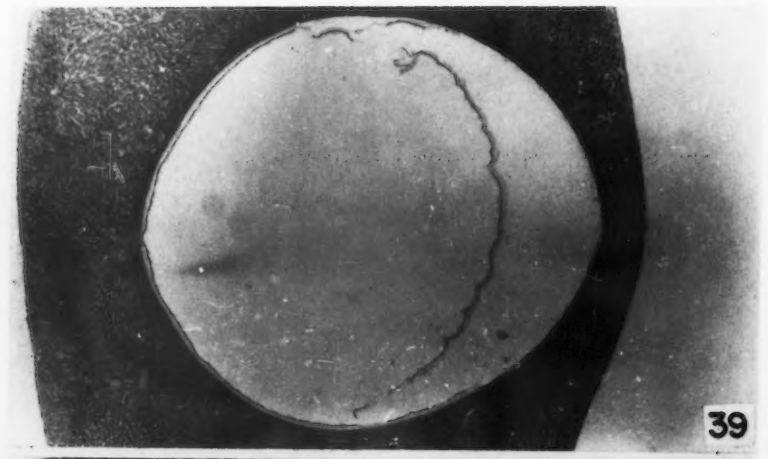


FIG. 39. A monovesicular cyst in a cotton rat infected with *E. granulosus* (Lebanon). The cyst is apparently healthy and growing and the host reaction is slight. (× 30)

FIG. 40. A killed cyst in the liver of a chinchilla infected with *E. g. canadensis*. The cuticle is greatly overgrown and convoluted and the fibrous layer is thick but not active. (× 30)

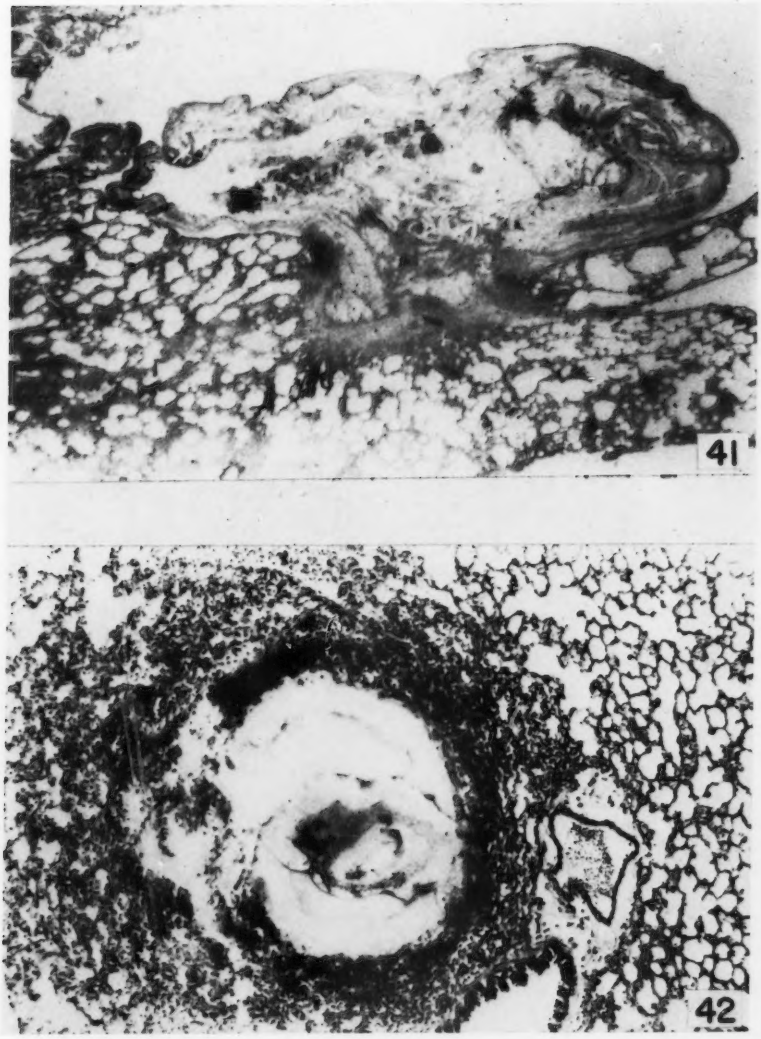


FIG. 41. A chinchilla, exposed to onchospheres of *E. g. canadensis*, developed small cysts in the lungs. The cysts were dead, the cuticle hypertrophied and folded, and the host reaction considerable. (× 12)

FIG. 42. A cyst in the lungs of an AKR mouse, resulting from onchospheres of *E. g. canadensis*, of similar appearance to that in the chinchilla. (× 30)

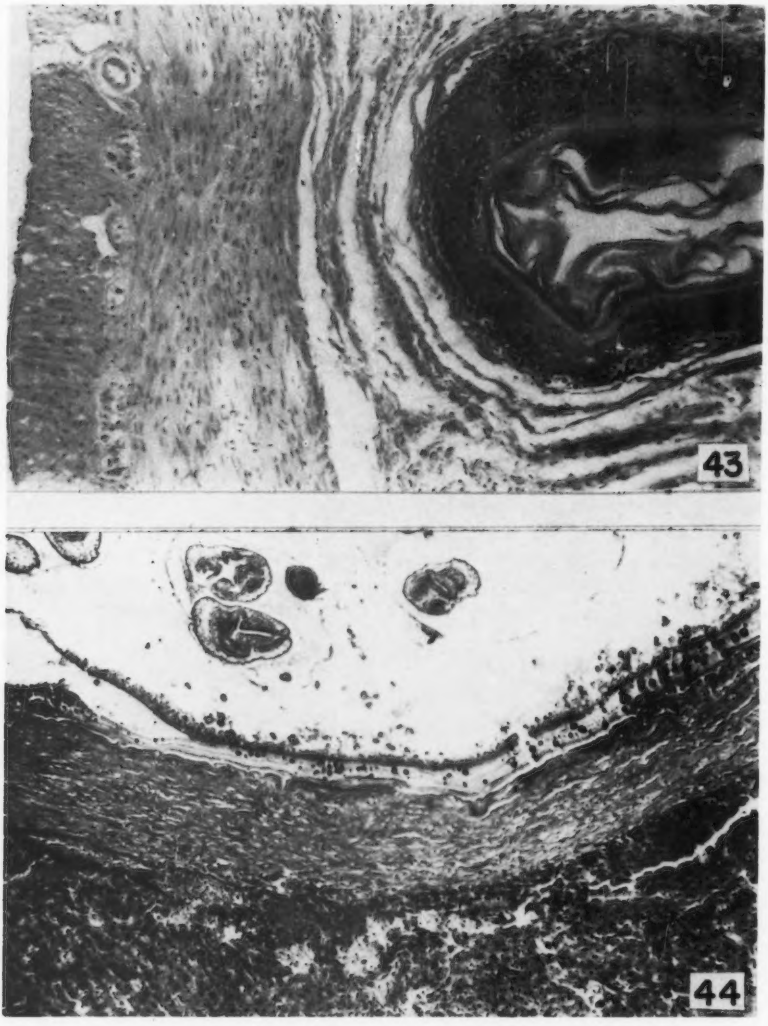


FIG. 43. Edge of a dead cyst on the liver of a golden hamster infected with *E. g. canadensis*. The cyst has excessive cuticular membrane and while the inner portion of the adventitia is dense and almost cartilaginous, the outer portion still shows an infiltration with leucocytes and fibroblasts. (× 120)

FIG. 44. A normal hydatid cyst in *Octodon*. This rodent was fed onchospheres of *E. granulosus* (Chile) which developed into a typical *granulosus* type cyst in the liver with a well-developed but relatively inactive fibrous adventitia. (× 75)

NOTES ON THE PARASITE COMPLEX OF EVAGORA (RECURVARIA) STARKI FREEMAN IN CANADIAN ROCKY MOUNTAIN PARKS[1]

R. W. STARK[2]

Abstract

The known parasites of the lodgepole needle miner, *Evagora* (*Recurvaria*) *starki* Freeman, 27 in all, are listed and notes on the biologies of the most common species are presented. Data are given to show fluctuations in parasite populations from 1944 to 1958. The probable reasons for the ineffectiveness of the parasites to control the needle miner are discussed briefly.

Introduction

The lodgepole needle miner, *Evagora* (*Recurvaria*) *starki* Freeman (6), is a defoliator of lodgepole pine, *Pinus contorta* ssp. *latifolia* (Engelm. ex Wats). It occurred in outbreak numbers in National Parks of Western Canada from about 1936 to 1950 and has been studied intensively since 1948. This paper, the author's fourth and final one in a series based on studies carried out from 1948 to 1959, deals with the species, numbers, and biologies of the parasites of the needle miner. The effect of the parasites on the population dynamics of the needle miner has already been discussed (25).

The Parasite Complex

Table I is a list of the known parasites of *E. starki* as determined by the Systematics Unit, Division of Entomology, Ottawa. The list is compiled from the Annual Reports of the Calgary Laboratory of Forest Biology, the Biological Control Laboratory, Belleville, Ontario, and numerous short publications (7, 9, 14, 20, 21, 22). The order of listing is taken from Muesebeck *et al.* (18).

Copidosoma deceptor Miller, *Apanteles starki* Mason, and *Apanteles californicus* Muesebeck comprise 90% or more of the total number of parasites present in field populations. The remaining species are comparatively rare and little known. Biological notes and comments on abundance are in order of importance.

Notes on Parasite Biologies

Apanteles

Muesebeck (18) states that all species of this genus seem to be internal parasites of lepidopterous larvae. At present, no method has been found to distinguish between the three species of *Apanteles* except in the adult stage. Determination of adults correlated with the sampling and rearing records provides clues to their distribution and abundance. The undetermined species (probably *A. miantonomoi* Viereck) is rare. *A. starki* (13) is the most numerous,

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TABLE I

Parasites of *Recurvaria starki* Freeman

Superfamily	Family	
ICHNEUMONOIDEA	Braconidae	1. *Apanteles californicus* Mues. 2. *A. starki* Mason 3. *A.* sp. poss. *miantonomoi* Vier. 4. *Eubadizon gracile* Prov. 5. *Meteorus pinifolii* Mason 6. *Bracon gelechiae* Ashm.
	Ichneumonidae	7. *Aethecerus pinifolii* Mason 8. *Alegina pinifoliae* (Cush.) 9. *Gelis tenellus* (Say) 10. *Itoplectis obesus* Cush. 11. *Phaedroctonus* sp. near *epinotiae* Cush. 12. *Phaeogenes* sp. near *epinotiae* Cush. 13. *Plectops* n. sp.
CHALCIDOIDEA	Eulophidae	14. *Dicladocerus westwoodii* West. 15. *Derostenus* sp. 16. *Euderus* sp. 17. *Neoderostenus* n. sp. 18. *Sympiesis guttatipennis* Grlt. 19. *Tetrastichus* sp. 20. *Zagrammosoma americanum* Grlt. 21. *Z. nigrolineatum* Cwfd.
	Encyrtidae	22. *Copidosoma deceptor* Miller
	Pteromalidae	23. *Amblymerus* sp. 24. *Habrocytus* sp. 25. *Pachyneuron* sp.
	Chalcididae	26. *Spilochalcis* sp. prob. *albifrons* (Walsh)
SPHECOIDEA	Sphecidae	27. *Passaloecus armeniacae* Ckll.

outnumbering *A. californicus* almost 8:1. *A. californicus* is the most widely distributed, occurring in all sampling locations in the Canadian outbreak including Jasper National Park and on other *Evagora* species in Oregon and California. *A. starki* is apparently restricted to the outbreak in Banff, Yoho, and Kootenay National Parks. The life history notes given probably describe *A. starki* mainly since they are the most abundant and most observations were made on material collected in the Banff area.

Emergence records for 1954 and 1958 show that *Apanteles* emerged in early July at about the same time as the needle miner; parasite emergence in 1956 was markedly earlier and occurred in two peak periods, June 23 and 28. After the first peak of emergence, cold, rainy weather persisted until June 27, the day before the second emergence peak. Emergence continued to about July 7. McLeod (14) recorded emergence of *Apanteles* in late July and August but this did not occur in the field in 1954, 1956, or 1958.

The emergence dates and life spans (about 3 weeks) of *Apanteles* suggest that they parasitize the egg stage of the needle miner. Morgan (17) states that *A. californicus* attacks the egg stage of *E. milleri* in California, but his evidence is also circumstantial.

The parasite is internal and apparently does not affect the normal feeding rate of its host until the latter reaches the fourth or fifth instar (Fig. 1, *a–c*).

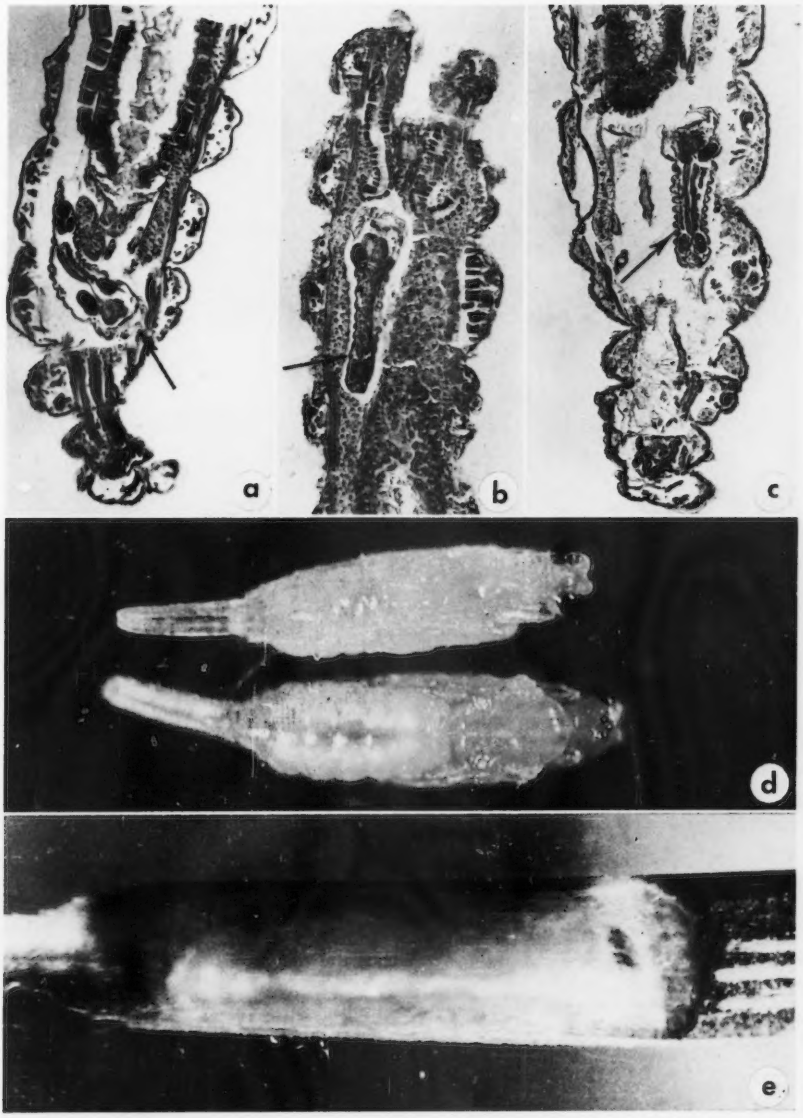

FIG. 1, *a–c*. Photomicrographs of *Apanteles* sp. (probably *starki*) in IV and V instar larvae: (*d*) pupae removed from cocoon; (*e*) cocoon of *Apanteles* sp.

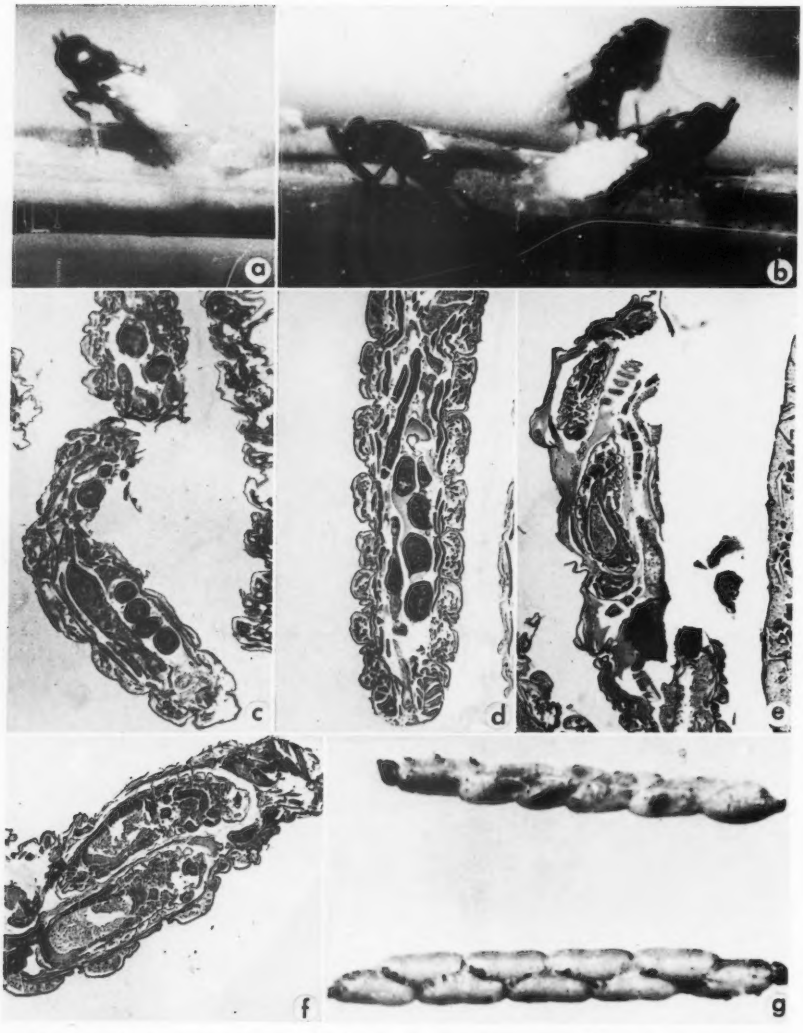

FIG. 2, *a–b*. Adult females of *Copidosoma deceptor* Miller ovipositing in needle miner eggs: e.g., developmental stages of *Copidosoma* in (*c*) III instar, (*d*, *e*) IV instar, and (*f*, *g*) V instar needle miner larva.

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Upon the death of the needle miner larva in April or May of the moth flight year, the parasite emerges from the host and spins a white, opaque, silken cocoon within and near the base of the mined needle (Fig. 1, *d*, *e*). This is the first visible evidence of parasitism by *Apanteles*. The emerging adult cuts a circular cap off the cocoon and crawls out of the exit hole prepared by the needle miner larva.

Sex ratios varied from year to year and from location to location but in total averaged almost a 1:1 ratio. There are at least two hyperparasites of *Apanteles*, *Alegina pinifoliae* (Cushman) and *Zagrammosoma nigrolineatum* Crawford. The hyperparasites may be polyembryonic or sometimes deposit more than one egg in the host as *Apanteles* cocoons had up to four emergence holes. The effect of hyperparasitism on *Apanteles* numbers has been negligible.

Copidosoma deceptor Miller

Miller described this species in 1958 and concluded that it was the only *Copidosoma* species attacking *E. starki*, *E. milleri*, and probably other *Evagora* species throughout western North America (16).

It is polyembryonic with as many as 14 adults emerging from a single host larva; the average from *E. starki* was about seven per host. Emergence occurred from July 15 to August 6 in 1954, July 11 to 30 in 1956, and July 5 to 28 in 1958, corresponding with the emergence and oviposition period of the host. There was a marked preponderance of females in some areas but the over-all sex ratio approximated 1:1.

When recently emerged adult females were placed in vials with mined needles containing host eggs they determined the location of eggs by tapping the needle with their antennae. If an egg cluster was beyond its reach from the exit hole, the adult female would either abandon the needle or search for some alternative access to the egg cluster. Several adults were seen to oviposit in the same eggs when placed in a vial with only one egg cluster. Individual females were seen ovipositing into the same egg several times (Fig. 2, *a*, *b*).

It is presumed that the development is similar to that described for *C. gelechiae* Howard (11). Early developmental stages of the parasite were not found in larval sections until the third instar of the needle miner (Fig. 2, *c*) and following this were easily recognizable in the fourth (Fig. 2, *d*, *e*) and fifth (Fig. 2, *f*) instars. As the needle miner does not reach the third instar until the second year of larval development, it is possible that the parasite overwinters in the polygerm stage, development progressing rapidly throughout the summer of the larval stages of the host. The parasites do not kill the host until it is in the fourth or fifth instar and do not become visible externally until the host is dead (Fig. 2, *g*).

Eubadizon gracile Provancher

Little is known of the subfamily (Blacinae) to which this parasite belongs. *E. gracile* is listed from New Brunswick to British Columbia in Canada, and Maine and California in the United States. Other hosts are *E. milleri*, *Recurvaria piceaella* Kearfott, *Dioryctria reniculella* (Grote), *Choristoneura fumiferana* (Clemens), *Archips* sp. (18), and the black-headed budworm, *Acleris variana* (Fernald) (Prebble, pers. comm.). It was widely distributed

throughout the outbreak area in small numbers but occasionally became locally abundant.

The parasite is internal and leaves the carcass of the fourth or fifth instar larva in late May of the moth flight year. It spins a tough, brown, translucent cocoon within the needle midway between the base of the mine and the exit hole. The needle miner larva is unaffected until early May. Parasite emergence has occurred as early as June 18 and as late as July 10. Specimens in captivity lived only a few weeks.

Sympiesis sp.

McLeod (14) reported this as an external parasite of needle miner larvae, relatively abundant in Alberta prior to 1951. The sampling program from 1948 to 1958 showed that it was scarce. Parasites believed to be *Sympiesis* sp. have been recovered from needle miner larvae and pupae and also from the important parasite, *Copidosoma deceptor* Miller. A *Sympiesis* sp. is recorded as an external parasite on late instar larvae of *E. milleri* (17, 26).

Zagrammosoma americanum Girault

This was one of the parasites recorded earliest from Alberta. It was found emerging from needle miner larvae and also from cocoons of *Copidosoma deceptor*. Since that time it has regularly appeared in mass rearings but in small numbers. Recent collections contained a second species, *Z. nigrolineatum* Crawford, which is parasitic on *Apanteles* spp. as well as needle miner. Two species of *Zagrammosoma* are recorded from California, *Z. americanum* and a new (?) species (17, 26).

Phaeogenes sp.

Members of the subfamily to which this species belongs (Cryptinae) have been described as internal parasites of Lepidoptera that oviposit into either the host larva or pupa, but always emerge from the pupa (18). The only note made on this rare species is that it was observed emerging from the needle miner pupa on August 4 and was extremely active. Depending on the length of adult life it could parasitize either needle miner eggs, first instar larvae, or an alternate host. There was some evidence to suggest that it may have a 1-year cycle.

Neoderostenus n. sp.

Neoderostenus n. sp. was reported as an important parasite by McLeod in 1951 (14) but sampling since that time indicates that it, as well as the other two eulophids, is relatively rare. It completes its development in the host pupa and may parasitize the larva in the same year, carrying over on alternate hosts. It forms the naked black pupa typical of eulophids. There are usually four to six parasites per pupa but whether this is due to polyembryony or superparasitism is not certain. It is also recorded as a parasite of *Copidosoma* species (14).

Notes on Parasite Population Fluctuations

Prior to 1948, estimates of parasitism were based on a limited number of samples (7, 8). The first reasonably accurate estimates were made in 1948 and for the succeeding two needle miner generations. Precise estimates, with a better understanding of the species involved, were made in 1954, 1956, and 1958.

Percentage of parasitism for all areas is presented in Table II and for specific areas in subsequent tables (Tables III and IV). The estimates in these tables are based upon the populations present just prior to the winter before the moth flight year and indicate only the percentage of parasites which reached maturity. Although the effective parasitism with relation to the surviving host population would have been more meaningful, the earlier data were not gathered in a manner which would permit this. Table II shows the trend of parasitism over the period of the outbreak. The life tables presented later are a basis for demonstrating effective parasitism in future studies. Two values are shown for the spring of 1950. Twelve per cent was calculated on the basis of the number of parasites visible in the spring. However, many of these were dead, presumably as a result of winter kill. The percentage surviving (i.e. emerging), and thus the percentage of parasitism, obtained from mass rearing material collected in the spring was 1.7%.

TABLE II

Percentage parasitism in moth flight years (all areas)

Year	Parasitism, %	Most important parasite species (in numbers)
1944	11	*Copidosoma deceptor* Miller
1946	10	—
1948	10	—
1950	12 to 1.7	*Copidosoma deceptor* Miller
1952	10	*Copidosoma deceptor* Miller
1954	18	*Copidosoma deceptor* Miller *Apanteles starki* Mason *Apanteles californicus* Mues.
1956	30	*Apanteles starki* Mason *Copidosoma deceptor* Miller *Apanteles californicus* Mues.
1958	21	*Apanteles starki* Mason *Copidosoma deceptor* Miller *Eubadizon gracile* (Prov.) *Apanteles californicus* Mues.

TABLE III

Percentage parasitism in moth flight years for four areas now under constant investigation

Area	1950	1952	1954	1956	1958
Mount Eisenhower	19–3.7	4+	20	39	25
Bankhead	11–1.9	17	16		
Girouard				32	29
Massive	13–0	—	10	26	15
Cathedral	0	22	14	12	15

Partial records are available for several areas which demonstrate the variability of parasitism within the general outbreak area. These are summarized for areas selected to correspond with the four areas now being continuously studied (Table III). Two estimates are given for the 1950 samples in this table also.

The distribution of the principal parasites by location and elevation in 1954 is of interest (Table IV). These data clearly show the importance of three of the parasite species. Although its occurrence is not wholly consistent throughout the outbreak area, *Apanteles* appears to be more numerous in the valley bottom than on the slopes. This suggests that this species is more cold-hardy than *Copidosoma deceptor*, or that there is a fundamental difference in conditions required for oviposition.

Supplementary tables for the critical spring period of the moth flight year for the four areas on a continuous study basis are presented in Tables V to VIII, which relate to Tables I to VIII in Stark, 1959 (25). It was demonstrated that when the parasite population was estimated on the same sample unit as the needle miner (number per branch tip) their numbers remained more or less

TABLE IV

Species composition and parasitism in all areas sampled in 1954

Location	Elev.	Parasitism*	By species, % of total parasitism		
			Copidosoma deceptor	*Apanteles starki* and *californicus*	Others
Mount Eisenhower	4800	—	—	—	—
	5300	1.1	39.0	59.0	2.0
	5800	3.2	86.0	13.5	0.5
	6300	3.7	83.0	16.5	0.5
Massive	4800	0.1	—	100.0	—
	5300	1.5	47.4	52.6	—
	5800	5.9	48.4	51.6	—
	6300	2.3	21.0	79.0	—
Cathedral	4500	—	—	—	—
	5000	1.1	53.9	45.1	1.0
	5500	3.1	40.4	58.6	1.0
Lake Louise	5000	—	—	—	—
	5500	0.8	81.0	18.5	0.5
	6000	0.3	95.3	4.2	0.5
	6500	0.1	92.6	3.6	3.8
Brewster Creek	5200	7.0	37.9	61.9	0.2
Baker Creek	6000	9.2	30.8	69.2	0
Cascade Valley	5500	3.9	60.0	39.7	0.3
Saskatchewan Crossing	4700	0.2	11.3	88.7	—
Hawk Creek	4000	1.2	83.4	16.6	—

*Number of parasites per 5-year branch tip; the sampling unit used for the lodgepole needle miner (Stark 1958 (23)).

constant throughout the period of the outbreak (25, Fig. 9). Absolute parasite numbers actually increased following the high needle miner mortality year (1949–50) but have decreased steadily since 1954 (Table IX). However, as the percentage parasitism generally increased (Table III) this decline must have been at a slower rate than that of the needle miner population.

Discussion

It is generally accepted that population growth follows a logistic curve where density increases until a point is reached at which the trend will be reversed and the rate of increase begins to decline. It is also generally assumed that this reversal is brought about in two ways: through new and more severely unfavorable processes coming into play at successively higher levels of density and by

TABLE V

Supplementary life tables showing mortality of late instar larvae and pupae, Mount Eisenhower*

x	$1x$	$d \times F$	dx	$100qx$
		1954–56		
Instars III–IV (1955–1956)	416	Climate—winter mortality	143	34.37
Instars IV–V	273	Parasitism		
		Copidosoma deceptor Miller	86	31.50
		Apanteles starki Mason, *Apanteles californicus* Mues.	68	24.90
		Eubadizon gracile (Prov.)	5	1.83
		Undetermined species	1	0.38
			160	58.61
		Unknown	8	2.93
			168	61.54
Pupae	105	Unknown—poss. climate	26	24.76
		Parasitism		
		Phaeogenes sp. and others Less than	1	0.45
			26+	25.21
		1956–58		
X_3, X_4—III and IV instar, July, 1957	261	Various	27	10.34
X_5—IV and V instar	234	*Copidosoma deceptor* Miller	40	17.09
		Apanteles starki Mason, *A. californicus* Mues.	51	21.79
		Eubadizon gracile (Prov.)	0.5	0.21
		Unknown spp.	3.5	1.50
			95	40.59
X_6—Pupa and adults	139	*Phaeogenes* sp.	8	3.42

*Table headings: x, stage at which sample is taken; $1x$, the number surviving at the beginning of the stage noted in the x column; $d \times F$, the mortality factor responsible for dx, the number dying within the interval between successive samples; $100qx$, percentage mortality.

an increase in the intensity of action of various individual density-dependent factors or processes (19). From the data presented it can be seen that the parasites of the needle miner did not conform to this population theory nor could any of the population damping mechanisms discussed by Klomp (10) be demonstrated as effective in this study.

It is postulated that the climatic effects which permitted the "release" of the needle miner population in the period 1936 to 1950 (25) did not benefit the parasite population to the same extent. The first effect considered was that the parasites suffered heavier mortality during the winters of the outbreak but at low needle miner populations the evidence suggests the opposite to be true. That is, the parasite population is decreasing at a slower rate than the needle miner. Clausen (2) recognized, particularly with introduced parasites, that differential mortality of parasites can be a limiting factor to parasite success. These species may not be able to withstand as low temperatures as their hosts. Dowden's work (5) is an outstanding example of these effects.

TABLE VI

Supplementary life tables showing mortality of late instar larvae and pupae, Massive Mountain

x	$1x$	$d \times F$	dx	$100qx$
		1954–56		
III–IV instars	465	Various	96	20.65
IV–V instars, May, June, 1956	369	Predation by birds	124	33.60
		Parasitism		
		Apanteles starki Mason, *Apanteles californicus* Mues.	66	17.89
		Copidosoma deceptor Miller	52	14.09
		Eubadizon gracile (Grov.)	3	0.81
		Undetermined species	2	0.54
			123	33.33
			247	66.94
Pupae June, 1956	122	Climate—desiccation?	30	24.84
Emerged	92			
		1956–58		
X_3, X_4—III and IV instar, July, 1957	151	Various	89	58.94
X_5—IV and V instar	62	*Copidosoma deceptor* Miller	13	20.97
		Apanteles starki Mason, *A. californicus* Mues.	13	20.97
		Eubadizon gracile (Prov.)	2	3.23
		Unknown spp.	1	1.61
			29	46.77
X_6—Pupa and adults	33	No parasitism		

Control of the oyster-shell scale by *Aphytis mytilaspidis* is usually effective in the mild-wintered Annapolis valley in Nova Scotia but is ineffective in New Brunswick where winter temperatures fall to −20° F or lower (12). The success of parasites of scale insects in California is limited by low temperatures and extreme temperature fluctuations in the winter (3, 4). Uvarov (28) reviews several studies which show a differential mortality of parasites at low temperatures. Some internal parasites are more sensitive to low temperatures than the host in which they exist. However, he also gives examples which show that low temperatures can favor parasite populations where the parasitized hosts are less susceptible to temperature extremes than non-parasitized ones. Blais *et al.* (1) found this to be true for parasites of the forest tent caterpillar. Unseasonably warm weather in May followed by several days of freezing temperatures caused high mortality of larvae but its principal parasite was unaffected.

A second limitation to parasite success is thought to be the differential effect of weather on developmental rates. Uvarov (28) gives ample evidence to show that host and parasite are very often unequally adjusted to normal climatic

TABLE VII

Supplementary life table showing mortality of late instar larvae and pupae, Mount Girouard

x	lx	$d \times F$	dx	$100qx$
		1954–56		
III–IV instars	896	Various	228	25.44
IV–V instars, May–June, 1956	668	Predation by birds	76	11.38
		Parasitism		
		Apantelcs starki Mason, *A. californicus* Mues.	228	34.13
		Copidosoma deceptor Miller	50	7.48
		Undetermined species	10	1.50
			288	43.11
		Unknown	41	6.14
			405	60.63
Pupae June, 1956	263	Climate—desiccation	56	21.29
		Parasitism—unidentified species	2	7.60
			58	28.89
Emerged	205			
		1956–58		
X_3, X_4—III and IV instar, July, 1957	219	Various	15	6.85
X_5—IV and V instar	204	*Apanteles starki* Mason, *A. californicus* Mues.	82	40.20
		Copidosoma deceptor Miller	22	10.78
		Eubadizon gracile (Prov.)	6	2.94
		Unknown spp.	1	0.49
			111	54.41
X_6—Pupa and adults	93	No parasitism		

conditions and will react to disturbances in different ways. Slight differences in rate of development may have a profound effect on the success of a parasite, particularly when the stage attacked is of short duration and the period of oviposition of the parasite is equally short. Thus, DeBach *et al.* (4) found that winter temperatures affected the rate of development of the parasite of a scale insect and disrupted the synchronization of parasite emergence with the stage attacked, resulting in markedly reduced parasitism. It has been shown above that the emergence dates of two of the major parasite species of the needle miner were markedly different in two different generations. If the assumption

TABLE VIII

Supplementary life table showing mortality of late instar larvae and pupae, Cathedral Mountain

x	$1x$	$d \times F$	dx	$100qx$
		1954–56		
III–IV instars	181	Various	117	64.64
IV–V instar, May–June, 1956	64	Parasitism		
		Apanteles starki Mason, *A. californicus* Mues.	17	25.56
		Copidosoma deceptor Miller	4	6.25
		Undetermined species	1	1.56
			22	24.37
Pupae				
June, 1956	42			
Emerged	42			
		1956–58		
X_3, X_4—III and IV instar, July, 1957	30	Various	5	16.67
X_5—IV and V instar	25	*Eubadizon gracile* (Prov.)	7.8	31.20
		Apanteles starki Mason, *A. californicus* Mues.	1.0	4.0
		Copidosoma deceptor Miller	0.6	2.4
		Unknown spp.	0.6	2.4
			10	40.0
X_6—Pupa and adults		No parasitism		

TABLE IX

Number of parasites per branch tip in moth flight years

Year	Mount Eisenhower	Bankhead–Girouard*	Massive	Cathedral
1950	3.3	0.30	—	—
1952	0.85	—	—	—
1954	2.36	3.84	6.7	1.4
1956	1.60	2.90	1.23	0.22
1958	0.95	1.11	0.29	0.1

*The sample location was changed from Bankhead to Girouard in 1956.

is correct that these species are parasites of the egg stage, then the numbers of these species would be expected to be less in 1958 and this proved to be the case in some areas.

Another possible factor, based solely on example and speculation, is that cold temperatures may reduce the fecundity and fertility of the parasite to a greater degree than its host. There is ample evidence to indicate that fecundity and fertility of insects are affected by external factors. This study has dealt largely with winter extremes but these effects may occur at any time during the life cycle (28). DeBach *et al.* found that the fecundity of a parasite of the California red scale was markedly reduced by detrimental effects of winter weather (4).

Thalenhorst (26) states that in nature, parasites and predators rarely attain their theoretical maximum efficiency and gives as a major reason for this that the hosts are rarely dispersed uniformly over an area, even under outbreak conditions. Differences in mobility and the 'searching ability' of the parasite may place the parasite at a distinct disadvantage. Environmental conditions during adult parasite activity may seriously limit their ability to search out the habitat of the stage attacked by them. So many variables are involved in determining the success of parasitism in comparison to that of the host that it is not surprising that parasites may be ineffective in controlling populations of the host insect.

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NOTES

ANALYSIS OF DISSOLVED NITROGEN IN FISH BLOOD

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An analytical procedure is described for the analysis of dissolved nitrogen in the blood of fish. This method was developed during an investigation into the dissolved nitrogen relationship of trout and their water environment, utilizing the extractor and gas analyzer as described by Scholander, Van Dam, Claff, and Kanwisher (1) (Mark and Co., Randolph, Mass.). The blood nitrogen microtechniques of Edwards, Scholander, and Roughton (2) and Sundes (3) were found difficult to apply under field conditions. Increasing the size of the blood sample to 0.4 ml enhanced accuracy but further increased the tendency of the blood to coagulate within the apparatus. This was overcome by the addition of a small quantity of saturated sodium chloride to the blood in the extractor. The transfer of reagents to the extractor by means of the KOH cup proved unsatisfactory. Instead the reagents were injected into the extractor by means of small hypodermic syringes. The hyposulphite solution proved difficult to prepare under field conditions and was replaced with commercially available oxygen absorber (Oxorbent, Burrell Corp., Pittsburgh, Pa.). In water analysis this oxygen absorber is incompatible with the alkaline citrate normally used in the gas analyzer. Alternatively the gas analyzer was filled with a saturated solution of sodium chloride as described by Hoar (4) for use in gas analysis.

Reagents

Heparin solution: 10 mg per ml of sodium heparin.

Occlusion fluid: saturated sodium chloride colored with methyl red indicator and barely acidified with concentrated hydrochloric acid.

Caprylic alcohol: (2-octanol).

Acid sulphate solution: 150 g anhydrous sodium sulphate dissolved in 500 ml of distilled water and acidified with 25 ml concentrated sulphuric acid.

Ferricyanide solution: 62.5 g potassium ferricyanide, 30.0 g potassium bicarbonate, and 2.5 g of saponin dissolved in 250 ml of water.

Potassium hydroxide solution: 100 g potassium hydroxide dissolved in water to a total volume of 500 ml.

Oxygen absorber: Oxorbent.

The acid sulphate and ferricyanide solutions are essentially those of Sundes (3) and the occlusion fluid that of Hoar (4). The oxygen absorber was stored under oil in a separatory funnel and delivered into the gas analyzer by means of a short length of polyethylene tubing. The other solutions were held in reagent bottles and permitted to equilibrate with air. The blood, caprylic

alcohol, saturated sodium chloride, and ferricyanide were injected into the extractor by means of 0.5- or 1.0-ml tuberculin syringes fitted with No. 16 needles and short lengths of polyethylene tubing, the 20% potassium hydroxide was injected into the extractor with a 5-ml syringe similarly fitted.

Technique

The extractor and gas analyzer were cleaned with acid permanganate or acid dichromate solution and rinsed thoroughly. The extractor was next flushed with 0.5 ml of acid sulphate solution by drawing that volume of solution into the analyzer then turning it tip up and running the plunger up and down. The acid sulphate was expelled and this step repeated, removing any air. The plunger was pushed home leaving the syringe extension filled with acid sulphate (approximately 0.1 ml).

The fish were stunned (but not killed) electrically and a blood sample obtained by heart puncture. The blood was drawn into a heparinized 1-ml syringe, care being taken to avoid any appreciable negative pressure. The blood sample was large enough to permit the transfer of 0.4 ml of blood and still leave 0.2 ml or more of blood in the syringe with the small volume of heparin from the needle. If any free gas appeared in the sample either from gas leaving solution in the blood or from air contamination, the sample was discarded.

Following transfer of the blood sample to the extractor, a small quantity of caprylic alcohol was necessary to reduce frothing. It was desirable to use the smallest amount possible. This was gauged by injecting the caprylic alcohol into the extractor to the point of bore enlargement 3/4 in. from the tip (a volume of approximately 0.015 ml) of the syringe extension.

The clotting problem was overcome by [illegible] 0.5 ml of occlusion fluid into the extractor after the caprylic alcohol. The tip of the extractor was closed with a rubber cap and the contents mixed by shaking for 1/2 minute. Promptness in the addition of the caprylic alcohol and sodium chloride to the blood in the extractor appeared to lessen further the tendency of the blood to form large clots.

The cap was removed and 0.3 ml of ferricyanide solution injected into the extractor. The cap was replaced and the extractor contents again shaken for 1/2 minute. The result was a gas phase (carbon dioxide, nitrogen, and oxygen) of 3.5 ml overlying 0.9 ml of liquid.

The carbon dioxide of the gas phase was reabsorbed by injecting 3.0 ml of 20% potassium hydroxide into the extractor. If the blood had broken up properly it settled to the bottom of the liquid in fine particles, floating clots indicated trapped gas bubbles, and the sample was discarded. The gas bubble was rolled back and forth once over the potassium hydroxide then ran up into the syringe extension.

The gas bubble was transferred to the gas analyzer which previously had been cleaned and filled with occlusion fluid. The gas bubble was drawn through the capillary into the analyzer barrel then slowly returned to the capillary. The excess fluid was removed from the cup and the end of analyzer, the length of the total gas column recorded, and the bubble returned to the barrel. Oxygen absorber was run into the cup and drawn through the analyzer until the barrel

was filled (approximately 0.3 ml). The gas bubble was rolled about in the absorber then slowly returned to the capillary. The length of the gas column, the temperature of the gas analyzer, and the barometric pressure were recorded.

Calculation

The nitrogen content was corrected to a dry gas at standard temperature and pressure and expressed as volumes %. The calculations were based on the original work of Peters and Van Slyke (5) and on the Scholander, Van Dam, Claff, and Kanwisher (1) description for water.

1. Blank determinations for nitrogen in the reagents were averaged and the mean value subtracted from the measured length of the sample nitrogen column. The average of 10 blanks was 12.8 mm. Thus for fish No. 1 the nitrogen column was

$$34.3 - 12.8 = 21.5 \text{ mm.}$$

2. The remaining gas column was corrected to a blood sample size of 1.0 ml by multiplying by the inverse of the sample size:

$$21.5 \times \frac{1.0}{0.4} = 53.75 \text{ mm.}$$

3. The length of the gas column in mm was converted to cu. mm with a calibration correction factor for the gas analyzer. In this case the value was very close to 0.283 thus:

$$53.75 \times 0.283 = 15.21 \text{ cu. mm.}$$

4. The recorded barometric pressure was temperature corrected from the table of values to be found in the Handbook of Chemistry and Physics (6). At 19° C and a barometric pressure of 767 mm Hg the corrected value was

$$767 - 2.4 = 764.6 \text{ mm Hg.}$$

5. The temperature-corrected barometric pressure was corrected to a dry gas by subtracting the water vapor pressure at the temperature of the gas analyzer (19° C):

$$764.6 - 16.5 = 748.1 \text{ mm Hg.}$$

6. The nitrogen volume as a dry gas was corrected to the standard pressure of 760 mm Hg:

$$15.21 \times \frac{748.1}{760} = 14.97 \text{ cu. mm.}$$

7. The nitrogen volume at standard pressure was corrected to the standard temperature of 0° C from the temperature (19° C) of the gas analyzer:

$$14.97 \times \frac{273}{292} = 14.00 \text{ cu. mm.}$$

8. A small correction was made for nitrogen remaining in solution in the syringe analyzer. The nitrogen in solution was a function of the partial pressure and solubility of the gas. The partial pressure of the nitrogen in the gas phase:

$$\frac{N_2 \text{ vol.}}{CO_2 + N_2 + O_2 \text{ vol.}} \times 760.$$

In the example at hand the nitrogen column in the gas analyzer was

$$34.3 \times 0.283 = 9.71 \text{ cu. mm.}$$

The total gas phase was approximately 3.5 ml or 3500 cu. mm. Thus the partial pressure of the nitrogen in the gas phase:

$$\frac{9.71}{3500} \times 760 = 2.1 \text{ mm Hg.}$$

The solubility of nitrogen at 19° C from the absorption coefficient, Handbook of Chemistry and Physics (6), was 15.70 cu. mm per ml of water. For a liquid phase of 0.9 ml at a partial pressure of 760 mm Hg:

$$15.70 \times 0.9 = 14.13 \text{ cu. mm.}$$

Correcting to the nitrogen partial pressure of 2.1 mm Hg (from above) the volume of nitrogen remaining in solution was

$$\frac{2.1}{760} \times 14.13 = 0.039 \text{ cu. mm.}$$

Thus the nitrogen volume of 9.71 cu. mm was increased by a factor of

$$\frac{9.71 \times 0.039}{9.71} = 1.004.$$

Correcting the volume from step No. 7, the nitrogen volume was

$$14.00 \times 1.004 = 14.06 \text{ cu. mm}$$

of dry gas at standard temperature and pressure for a blood sample of 1.0 ml, or 1.41 volumes %.

9. Theoretical values were calculated from the table of absorption coefficients, Handbook of Chemistry and Physics (6). NOTE: the absorption coefficient should not be confused with the coefficient of solubility, the latter being calculated to the temperature and pressure at which the gas is measured. A fuller explanation may be found in Glasstone (7).

At the sample temperature of 11.6° C the solubility of dry nitrogen reduced to S.T.P. was 18.01 ml per liter, obtained by interpolation between values in the table of absorption coefficients. The recorded barometric pressure was temperature corrected as above:

$$767 - 2.4 = 764.6 \text{ mm Hg.}$$

This corrected pressure was reduced to conditions of a dry gas by subtracting the water vapor pressure at the temperature of the sample:

$$764.6 - 10.2 = 754.4 \text{ mm Hg.}$$

In dry air the partial pressure of nitrogen (the noble gases in the air were also considered as nitrogen here) was

$$0.7905 \times 754.4 = 596.4 \text{ mm Hg.}$$

Correcting the absorption coefficient value to this nitrogen partial pressure, at 11.6° C the theoretical nitrogen content of air-equilibrated water was

$$18.01 \times \frac{596.4}{760} = 14.14 \text{ ml per liter}$$

or 1.41 volumes %.

10. The percentage of air saturation was calculated from the corrected experimental value, No. 8, and the corrected theoretical nitrogen, No. 9:

$$\frac{14.06}{14.14} \times 100 = 99.4\%.$$

11. Corrections were not made for certain lesser inaccuracies. There was a difference in the size of the liquid phase and hence a difference in the amount of nitrogen remaining in solution in the blank and blood determinations. The solubility of nitrogen in the reagents of the liquid phase was less than that of the value for water used in the calculation No. 8. This was opposed by the higher solubility of nitrogen in blood than in water. A measure of this may be found in Van Slyke, Dillon, and Margaria (8).

Accuracy

Ten blank determinations averaged 12.8 mm with one standard deviation ± 0.4 mm. The average of 10 determinations on distilled water, air-equilibrated in a rotating flask was 101.0% of air saturation with $\pm 2.1\%$ for one standard deviation. Ten blood determinations on rainbow trout held in a pond at 99–101% of air saturation averaged 99.4% with $\pm 2.8\%$ for one standard deviation.

The technique described was employed successfully over the range of 100 to 135% of air saturation (1.31 to 1.77 volumes %) in a study of the rate of change of dissolved nitrogen in the blood of trout. The results of this study will be discussed in relation to physical and limnological supersaturation in a later report.

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THE GENUS MANISTRONGYLUS—A CORRECTION

THOMAS W. M. CAMERON AND BETTY J. MYERS

In a recent publication (2) we established the genus *Manistrongylus* for a trichostrongyle previously described as *Trichostrongylus meyeri* Travassos, 1937 from *Manis pentadactyla* from Formosa. At the time we were unaware of Baer's publication (1) in which he established the genus *Manistrongylus* for a species of trichostrongyle from *Manis tricuspis* from Africa. In this publication Baer proposed the new combination *Manistrongylus meyeri* for *Trichostrongylus meyeri* Travassos, 1937. Therefore, his generic name—*Manistrongylus* Baer, 1959—has precedence, and our genus, *Manistrongylus* Cameron & Myers, 1960, becomes a homonym. The former *T. meyeri* Travassos, 1937 should now be called *Manistrongylus meyeri* (Travassos, 1937) Baer, 1959.

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